

Supplementary Information:

Complex modular architecture around a simple toolkit of wing pattern genes

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S1. Color pattern loci

Table S 1. Summary of major loci affecting color pattern variation in *Heliconius erato* considered in this study and their original description.

Locus	Major phenotypic effect	Original description of phenotype (Sheppard et al. 1985)	Candidate Gene
<i>Sd</i>	Forewing band shape	Shortened: affecting shape of the forewing band	<i>wntA</i>
<i>St</i>	Forewing band shape	Split: breaking up forewing band in two spots	<i>wntA</i>
<i>Ly</i>	Forewing band shape	Linked to yellow line: breaking up forewing band in multiple spots	<i>wntA</i>
<i>Ro</i>	Forewing band shape	Round: affecting shape distal portion of forewing band	<i>vvl</i> or <i>rsp3</i> *
<i>R</i>	Red pattern	Rays: red rays on hindwing	<i>optix</i>
<i>D</i>	Red pattern	Dennis: red patch in proximal part of forewing	<i>optix</i>
<i>Y</i>	Red pattern	Band: red forewing band	<i>optix</i>
<i>Cr</i>	Yellow hindwing band	Cream rectangles: yellow hindwing bar (and white hindwing fringes in <i>H. e. cyrbia</i>)	<i>cortex</i>

*In this study we identify two likely gene candidates underlying variation at *Ro*.

S2. Genome assembly

S2.1. Library construction and sequencing

Table S 2. Sequencing data for *Heliconius erato (demophoon)* genome assembly and linkage mapping.

	Insert size	Sample	Intrinsic coverage	Type	Sequencing Center	SRA accession
Small insert pair-end sequencing	250	STRI_6581	127	PE 150	BGI	SAMN05578372
	500	STRI_6581	52	PE 150	BGI	SAMN05578373
	800	STRI_6581	43	PE 100	BGI	SAMN05578374
Small mate-pair bridging libraries and sequencing	4.3kb	STRI_6582	61	PE 100	Duke	SAMN05578375
	6.5kb	STRI_6582	46	PE 100	Duke	SAMN05578376
PacBio Long Reads	-	STRI_6591	25	-	Duke	SAMN05578377
Mapping Family	250	STRI_6405 (Father)	30	PE 100	Duke	SAMN05572290
	250	STRI_6659 (Mother)	47	PE 100	Duke	SAMN05572291
	250	STRI_6520	9	PE 100	Duke	SAMN05572292
	250	STRI_6523	10	PE 100	Duke	SAMN05572293
	250	STRI_6524	12	PE 100	Duke	SAMN05572294
	250	STRI_6525	9	PE 100	Duke	SAMN05572295
	250	STRI_6526	11	PE 100	Duke	SAMN05572296
	250	STRI_6527	7	PE 100	Duke	SAMN05572297
	250	STRI_6528	8	PE 100	Duke	SAMN05572298
	250	STRI_6529	6	PE 100	Duke	SAMN05572299
	250	STRI_6530	9	PE 100	Duke	SAMN05572300
	250	STRI_6531	8	PE 100	Duke	SAMN05572301
	250	STRI_6532	8	PE 100	Duke	SAMN05572302
	250	STRI_6533	8	PE 100	Duke	SAMN05572303
	250	STRI_6534	9	PE 100	Duke	SAMN05572304
	250	STRI_6539	11	PE 100	Duke	SAMN05572305
	250	STRI_6542	8	PE 100	Duke	SAMN05572306
	250	STRI_6543	9	PE 100	Duke	SAMN05572307
	250	STRI_6544	7	PE 100	Duke	SAMN05572308
	250	STRI_6550	9	PE 100	Duke	SAMN05572309
	250	STRI_6551	8	PE 100	Duke	SAMN05572310
	250	STRI_6562	10	PE 100	Duke	SAMN05572311
	250	STRI_6619	8	PE 100	Duke	SAMN05572312
	250	STRI_6620	9	PE 100	Duke	SAMN05572313
	250	STRI_6643	8	PE 100	Duke	SAMN05572314
	250	STRI_6644	10	PE 100	Duke	SAMN05572315
	250	STRI_6645	12	PE 100	Duke	SAMN05572316
	250	STRI_6646	10	PE 100	Duke	SAMN05572317
	250	STRI_6660	10	PE 100	Duke	SAMN05572318
	250	STRI_6661	8	PE 100	Duke	SAMN05572319
	250	STRI_6662	9	PE 100	Duke	SAMN05572320
	250	STRI_6663	11	PE 100	Duke	SAMN05572321
	250	STRI_6664	5	PE 100	Duke	SAMN05572322
	250	STRI_6563	6	PE 100	Duke	SAMN05572323
	250	STRI_6564	6	PE 100	Duke	SAMN05572324
	250	STRI_6565	16	PE 100	Duke	SAMN05572325
	250	STRI_6566	7	PE 100	Duke	SAMN05572326
	250	STRI_6567	12	PE 100	Duke	SAMN05572327
	250	STRI_6568	10	PE 100	Duke	SAMN05572328
	250	STRI_6569	9	PE 100	Duke	SAMN05572329
	250	STRI_6570	13	PE 100	Duke	SAMN05572330
	250	STRI_6576	13	PE 100	Duke	SAMN05572331
	250	STRI_6577	14	PE 100	Duke	SAMN05572332

250	STRI_6578	11	PE 100	Duke	SAMN05572333
250	STRI_6579	9	PE 100	Duke	SAMN05572334
250	STRI_6580	8	PE 100	Duke	SAMN05572335
250	STRI_6583	10	PE 100	Duke	SAMN05572336
250	STRI_6584	8	PE 100	Duke	SAMN05572337
250	STRI_6585	9	PE 100	Duke	SAMN05572338
250	STRI_6586	10	PE 100	Duke	SAMN05572339
250	STRI_6592	8	PE 100	Duke	SAMN05572340
250	STRI_6593	8	PE 100	Duke	SAMN05572341
250	STRI_6594	10	PE 100	Duke	SAMN05572342
250	STRI_6601	11	PE 100	Duke	SAMN05572343
250	STRI_6602	9	PE 100	Duke	SAMN05572344
250	STRI_6606	9	PE 100	Duke	SAMN05572345
250	STRI_6712	8	PE 100	Duke	SAMN05572346
250	STRI_6713	10	PE 100	Duke	SAMN05572347
250	STRI_6714	9	PE 100	Duke	SAMN05572348
250	STRI_6715	9	PE 100	Duke	SAMN05572349
250	STRI_6716	8	PE 100	Duke	SAMN05572350
250	STRI_6726	10	PE 100	Duke	SAMN05572351
250	STRI_6727	9	PE 100	Duke	SAMN05572352
250	STRI_6728	10	PE 100	Duke	SAMN05572353
250	STRI_6744	13	PE 100	Duke	SAMN05572354
250	STRI_6745	7	PE 100	Duke	SAMN05572355
250	STRI_6746	10	PE 100	Duke	SAMN05572356
250	STRI_6751	9	PE 100	Duke	SAMN05572357
250	STRI_6752	10	PE 100	Duke	SAMN05572358
250	STRI_6753	8	PE 100	Duke	SAMN05572359
250	STRI_6754	8	PE 100	Duke	SAMN05572360
250	STRI_6755	8	PE 100	Duke	SAMN05572361
250	STRI_6767	10	PE 100	Duke	SAMN05572364
250	STRI_6768	10	PE 100	Duke	SAMN05572365
250	STRI_6769	7	PE 100	Duke	SAMN05572366
250	STRI_6782	9	PE 100	Duke	SAMN05572367
250	STRI_6783	11	PE 100	Duke	SAMN05572368
250	STRI_6784	11	PE 100	Duke	SAMN05572369
250	STRI_6787	10	PE 100	Duke	SAMN05572372
250	STRI_6816	7	PE 100	Duke	SAMN05572373
250	STRI_6477	10	PE 100	Duke	SAMN05572374
250	STRI_6478	8	PE 100	Duke	SAMN05572375
250	STRI_6495	11	PE 100	Duke	SAMN05572376
250	STRI_6501	10	PE 100	Duke	SAMN05572377
250	STRI_6502	10	PE 100	Duke	SAMN05572378
250	STRI_6506	10	PE 100	Duke	SAMN05572379
250	STRI_6500	8	PE 100	Duke	SAMN05572380
250	STRI_6503	10	PE 100	Duke	SAMN05572381
250	STRI_6505	9	PE 100	Duke	SAMN05572382
250	STRI_6493	9	PE 100	Duke	SAMN05572383
250	STRI_6494	9	PE 100	Duke	SAMN05572384
250	STRI_6496	12	PE 100	Duke	SAMN05572385
250	STRI_6497	9	PE 100	Duke	SAMN05572386
250	STRI_6498	10	PE 100	Duke	SAMN05572387
250	STRI_6499	12	PE 100	Duke	SAMN05572388
250	STRI_6610	9	PE 100	Duke	SAMN05572389
250	STRI_6611	18	PE 100	Duke	SAMN05572390

S2.2. *De novo* assemblies

Table S 3. *De novo* genome assemblies.

Assembly	Data	Length	N50	Scaffolds	%Gaps
Herato_stage1	Illumina/Pacbio	402,810,510	611,657	1,275	4.100
Herato_stage2	Linkage map corrected	404,432,779	805,173	746	4.100
Herato_stage3	Discovar/Allpaths correctd	382,844,248	10,745,484	196	1.400

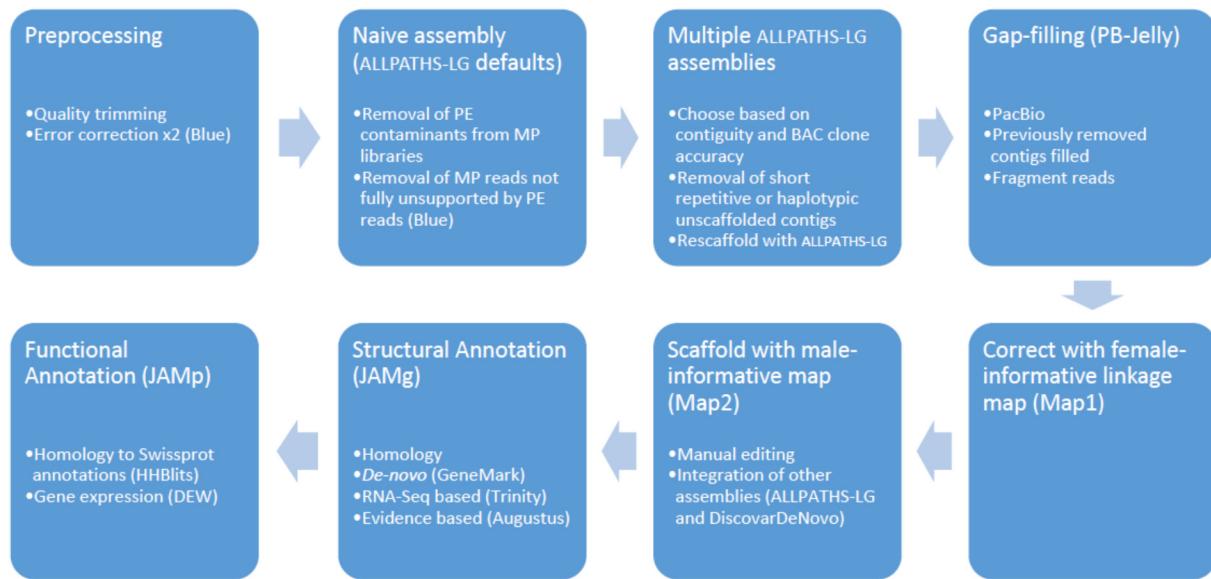


Figure S 1. Schematic figure of *Heliconius erato* genome assembly and annotation process^{1–5}.

S2.3. Linkage mapping and assembly validation

Table S 4. Linkage map statistics. Two maps were constructed from markers informative only on the mother (map 1) or father (map 2). We used biphasic linkage mapping⁶: achiasmatic meiosis in Lepidoptera, the maternal map only gives information on the chromosome of markers and not the position within each chromosome.

Chromosome	Markers in map 1	Markers in map 2	Map 1 length	Mb
1	138070	116509	49	23.3
2	106156	0	38	14.3
3	78332	68566	47	17.8
4	76837	71440	48	17.3
5	81039	74708	44	14.5
6	148236	65212	44	19.8
7	179873	23584	42	19.7
8	92129	41707	40	15.3
9	64311	47910	42	14.5
10	166114	120991	47	24.5
11	100043	84406	45	18.3
12	170326	109476	50	21.8
13	174551	75580	44	24.7
14	75735	69027	39	15.7
15	104692	50336	52	18
16	87143	75736	47	19
17	107757	84390	36	21.4
18	152173	94396	52	22.8
19	129946	96628	47	23.6
20	112258	81843	52	20.4
Z	32676	15769	41	18.4
Total	2378397	1468214	946	405.1*

*The total genome length is slightly bigger than the total length of the Herato_stage2 assembly as short errors and uncertain regions are sometimes put to multiple chromosomes. The final Herato_stage3 assembly (< 400Mb) was obtained by resolving these and other uncertain regions.

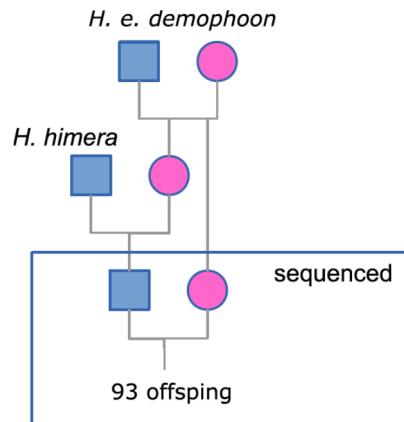


Figure S 2. Backcross for linkage mapping.

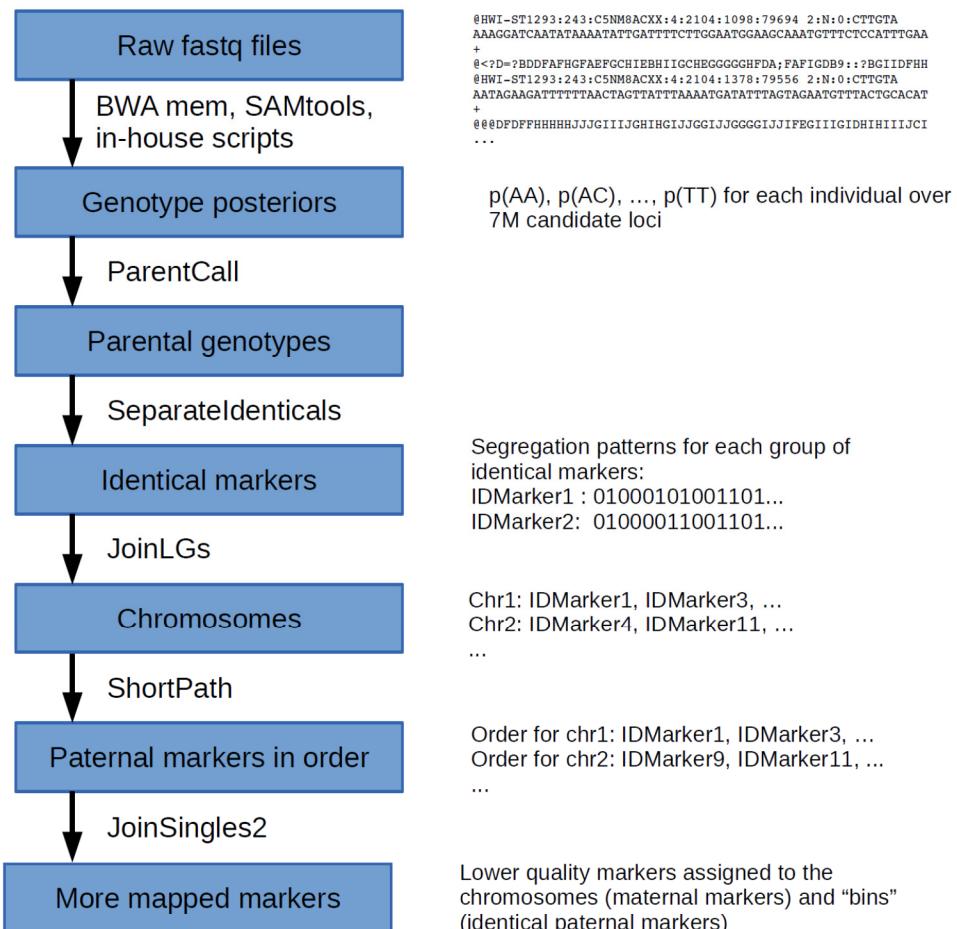


Figure S 3. Schematic figure of linkage mapping process using Lep-MAP3.

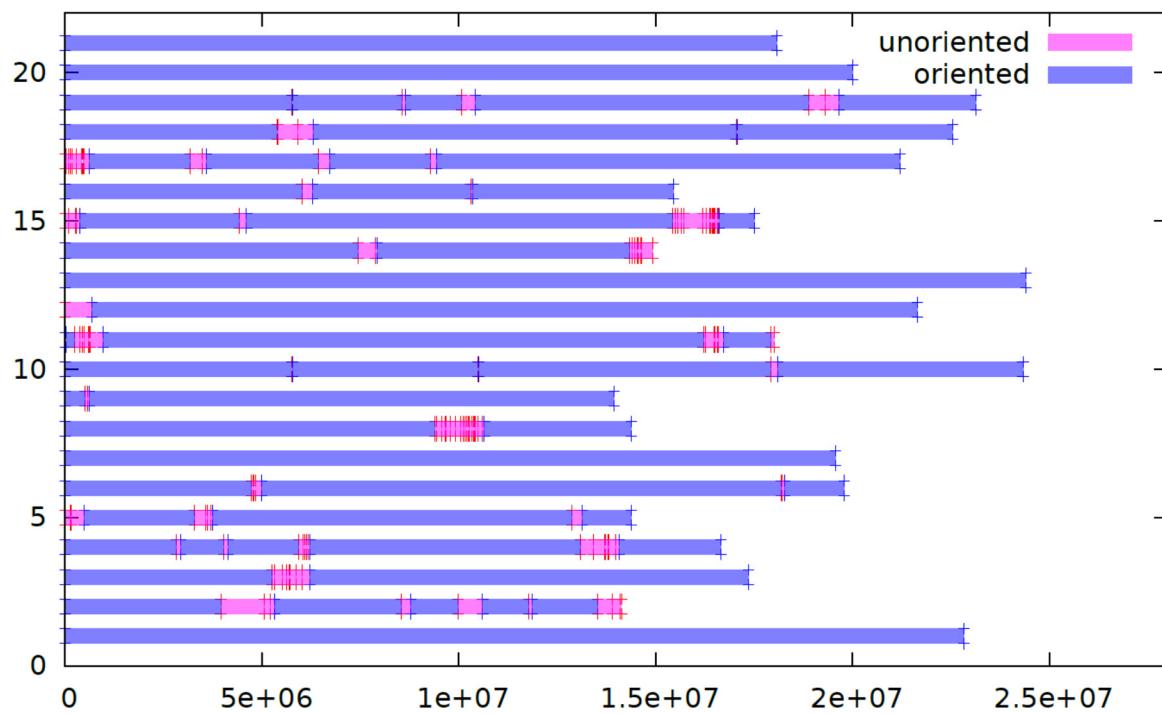
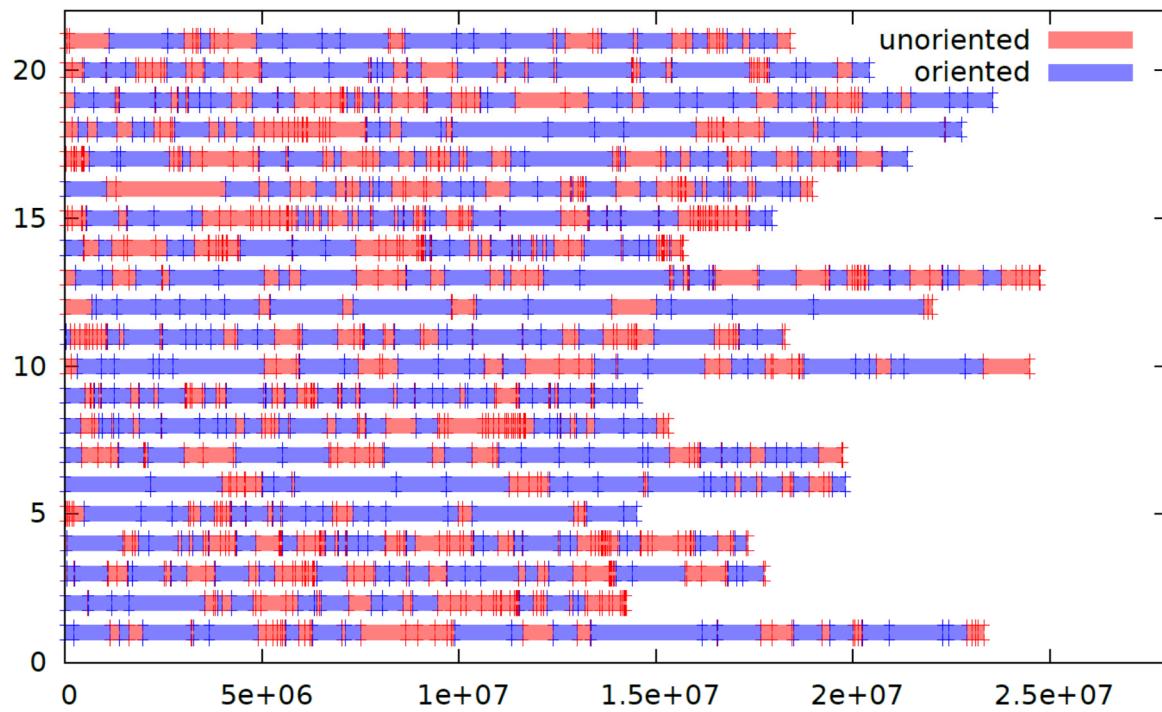


Figure S 4. Genome assembly results. Upper figure shows the completeness of the chromosomes in the assembly after made error-free based on the linkage maps (Herato_stage2). Below is the final assembly after removing haplotypic scaffolds and joining scaffold based on secondary genome assemblies and PacBio data (Herato_stage3).

S2.4. *H. erato* and Lepidoptera genome assembly statistics

Assembly statistics for the *Heliconius erato* and other Lepidoptera genomes were computed using summarizeAssembly.py in PBSuite 14.9.9⁷. Assembly completeness was assessed against a benchmarked set of 2,675 single-copy orthologs using BUSCO⁸.

Table S 5. Lepidoptera genome assembly statistics. Assembly size and scaffold statistics

	Scaffolds	Total length	Mean scaffold size	Maximum scaffold size
<i>Heliconius erato</i>	196	382,844,248	1,953,287	23,854,410
<i>Heliconius melpomene</i> v1	4,309	273,786,188	63,538	1,451,426
<i>Heliconius melpomene</i> v2	795	275,198,613	346,161	9,352,983
<i>Bombyx mori</i>	43,462	481,803,763	11,085	16,203,812
<i>Danaus plexippus</i>	5,397	248,564,116	46,055	6,243,218
<i>Lerema accius</i>	29,988	298,173,436	9,943	3,082,282
<i>Melitaea cinxia</i>	8,261	389,907,520	47,198	668,473
<i>Papilio glaucus</i>	68,029	375,987,417	5,526	1,977,235
<i>Papilio polytes</i>	3,873	227,005,758	58,612	9,881,032
<i>Papilio xuthus</i>	5,572	243,890,167	43,770	16,292,344
<i>Plutella xylostella</i>	1,819	394,062,517	216,636	3,493,687
<i>Bicyclus anynana</i>	15,180	458,610,584	30,211	2,943,548
<i>Chilo suppressalis</i>	80,479	372,375,373	4,626	111,673
<i>Manduca sexta</i>	20,871	419,424,771	20,096	3,253,989
<i>Plodia interpunctella</i>	10,542	381,952,380	36,231	7,207,896

Table S 6. Lepidoptera genome assembly statistics. Scaffold statistics.

	Scaffold N50	Scaffold N90	Scaffold N95	Scaffold L50	Scaffold L90	Scaffold L95
<i>Heliconius erato</i>	10,745,484	2,677,609	1,104,102	12	40	52
<i>Heliconius melpomene</i> v1	194,302	38,051	21,864	345	1,634	2,105
<i>Heliconius melpomene</i> v2	2,102,720	273,111	124,798	34	176	251
<i>Bombyx mori</i>	4,008,358	61,147	928	38	258	5,679
<i>Danaus plexippus</i>	715,606	160,499	68,064	101	366	483
<i>Lerema accius</i>	525,349	60,308	1,913	160	689	3,385
<i>Melitaea cinxia</i>	119,328	29,598	16,097	970	3,396	4,263
<i>Papilio glaucus</i>	230,299	2,022	945	421	7,589	21,037
<i>Papilio polytes</i>	3,672,263	930,396	417,439	21	63	81
<i>Papilio xuthus</i>	6,198,915	533,617	160,478	16	48	91
<i>Plutella xylostella</i>	737,182	152,088	72,492	155	575	753
<i>Bicyclus anynana</i>	364,913	55,873	22,189	303	1,523	2,131
<i>Chilo suppressalis</i>	5,215	2,401	2,191	19,910	63,459	71,579
<i>Manduca sexta</i>	664,006	46,417	4,807	169	1,025	2,265
<i>Plodia interpunctella</i>	1,270,674	18,727	8,920	76	713	2,348

Table S 7. Lepidoptera genome assembly statistics. Contigs and gabs.

	Contigs	Mean contig size	Contig N50	Gaps	Total gap length	Gap %
<i>Heliconius erato</i>	5,851	64,539	169,806	5,655	5,223,833	1.4
<i>Heliconius melpomene</i> v1	11,607	23,231	51,611	7,298	4,132,701	1.5
<i>Heliconius melpomene</i> v2	3,105	88,314	330,037	2,310	981,612	0.4
<i>Bombyx mori</i>	87,972	4,907	15,765	44,510	50,083,569	10.4
<i>Danaus plexippus</i>	10,545	22,939	113,903	5,148	6,664,276	2.7
<i>Lerema accius</i>	52,985	5,466	18,018	22,997	8,535,705	2.9
<i>Melitaea cinxia</i>	45,618	7,914	15,003	37,357	28,877,732	7.4
<i>Papilio glaucus</i>	96,532	3,754	12,958	28,503	13,599,067	3.6
<i>Papilio polytes</i>	13,441	16,239	51,561	9,568	8,725,522	3.8
<i>Papilio xuthus</i>	10,483	22,697	133,779	4,911	5,949,704	2.4
<i>Plutella xylostella</i>	15,764	24,557	59,184	13,945	6,937,203	1.8
<i>Bicyclus anynana</i>	28,866	15,650	60,000	13,686	6,852,021	1.5
<i>Chilo suppressalis</i>	331,320	983	2,183	250,841	46,484,604	12.5
<i>Manduca sexta</i>	35,212	11,351	51,909	14,341	19,705,457	4.7
<i>Plodia interpunctella</i>	17,231	21,160	338,910	6,689	17,329,456	4.5

Table S 8. Lepidoptera genome assembly statistics. BUSCO results.

	Complete Single-Copy BUSCOs %	Complete Duplicated BUSCOs %	Fragmented BUSCOs %	Missing BUSCOs %
<i>Heliconius erato</i>	82	2.3	11	6.3
<i>Heliconius melpomene</i> v1	81	2.8	11	7.2
<i>Heliconius melpomene</i> v2	85	3.1	9.4	5
<i>Bombyx mori</i>	75	2.2	16	8.4
<i>Danaus plexippus</i>	87	3.5	10	2.7
<i>Lerema accius</i>	77	2.6	13	8.3
<i>Melitaea cinxia</i>	55	1.6	20	23
<i>Papilio glaucus</i>	75	2.7	14	9.6
<i>Papilio polytes</i>	76	2.4	12	11
<i>Papilio xuthus</i>	84	3	8.2	7.4
<i>Plutella xylostella</i>	74	20	11	13
<i>Bicyclus anynana</i>	81	3	12	6.5
<i>Chilo suppressalis</i>	33	0.7	17	48
<i>Manduca sexta</i>	81	4.4	11	6.4
<i>Plodia interpunctella</i>	85	3.4	9.4	4.7

S3. RNAseq for genome annotation

Table S 9. Tissue and developmental stages for which RNA was sequenced for genome annotation.

Label	Developmental stage	Tissue	Sex	Race	SRA accession
emma_1d_pupae_hindwing_1	1d_pupae	hindwing	unknown	<i>emma</i>	SRR616680
emma_1d_pupae_hindwing_2	1d_pupae	hindwing	unknown	<i>emma</i>	SRR616681
emma_1d_pupae_hindwing_3	1d_pupae	hindwing	unknown	<i>emma</i>	SRR616682
emma_3d_pupae_hindwing_1	3d_pupae	hindwing	unknown	<i>emma</i>	SRR616686
emma_3d_pupae_hindwing_2	3d_pupae	hindwing	unknown	<i>emma</i>	SRR616687
emma_3d_pupae_hindwing_3	3d_pupae	hindwing	unknown	<i>emma</i>	SRR616688
emma_5th_instar_hindwing_1	5th_instar	hindwing	unknown	<i>emma</i>	SRR616278
emma_5th_instar_hindwing_2	5th_instar	hindwing	unknown	<i>emma</i>	SRR616674
emma_5th_instar_hindwing_3	5th_instar	hindwing	unknown	<i>emma</i>	SRR616675
favorinus_1d_pupae_hindwing_1	1d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616683
favorinus_1d_pupae_hindwing_2	1d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616684
favorinus_1d_pupae_hindwing_3	1d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616685
favorinus_3d_pupae_hindwing_1	3d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616689
favorinus_3d_pupae_hindwing_2	3d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616690
favorinus_3d_pupae_hindwing_3	3d_pupae	hindwing	unknown	<i>favorinus</i>	SRR616691
favorinus_5th_instar_hindwing_1	5th_instar	hindwing	unknown	<i>favorinus</i>	SRR616677
favorinus_5th_instar_hindwing_2	5th_instar	hindwing	unknown	<i>favorinus</i>	SRR616678
favorinus_5th_instar_hindwing_3	5th_instar	hindwing	unknown	<i>favorinus</i>	SRR616679
hyd_36h_larval_hw_prox	36h_larva	hindwing_proximal	unknown	<i>hydara</i>	SAMN05578182
hyd_prepupae_wing	prepupae	wing	unknown	<i>hydara</i>	SAMN05578183
pet_1d_pupa	1d_pupae	body	unknown	<i>petiverana</i>	SAMN05578184
pet_1instar	1st_instar	whole	unknown	<i>petiverana</i>	SAMN05578185
pet_24h_eggs	24h_egg	eggs	unknown	<i>petiverana</i>	SAMN05578186
pet_2instar	2nd_instar	body	unknown	<i>petiverana</i>	SAMN05578187
pet_3.5d_pupa	3.5d_pupae	body	unknown	<i>petiverana</i>	SAMN05578188
pet_36h_larval_hw_distal	36h_larva	hindwing_distal	unknown	<i>petiverana</i>	SAMN05578189
pet_36h_larval_hw_prox	36h_larva	hindwing_proximal	unknown	<i>petiverana</i>	SAMN05578190
pet_3instar	3rd_instar	whole	unknown	<i>petiverana</i>	SAMN05578191
pet_48h_eggs	48h_egg	eggs	unknown	<i>petiverana</i>	SAMN05578192
pet_4instar	4th_instar	body	unknown	<i>petiverana</i>	SAMN05578193
pet_4instar_gut	4th_instar	gut	unknown	<i>petiverana</i>	SAMN05578194
pet_5d_pupa	5d_pupae	body	unknown	<i>petiverana</i>	SAMN05578195
pet_5instar	5th_instar	body	unknown	<i>petiverana</i>	SAMN05578196
pet_9d_pupa	9d_pupae	body	unknown	<i>petiverana</i>	SAMN05578197
pet_eggs_12h	12h_egg	eggs	unknown	<i>petiverana</i>	SAMN05578198
pet_female_abdomen	adult	abdomen	female	<i>petiverana</i>	SAMN05578199
pet_female_eye	adult	eye	female	<i>petiverana</i>	SAMN05578200
pet_female_head	adult	head	female	<i>petiverana</i>	SAMN05578201
pet_female_thorax	adult	thorax	female	<i>petiverana</i>	SAMN05578202
pet_male_abdomen	adult	abdomen	male	<i>petiverana</i>	SAMN05578203
pet_male_head	adult	head	male	<i>petiverana</i>	SAMN05578204
pet_male_thorax	adult	thorax	male	<i>petiverana</i>	SAMN05578205
pet_prepupae_wing	prepupae	wing	unknown	<i>petiverana</i>	SAMN05578206

S4. Population genomics

S4.1. Sampling and sequencing

To determine the pattern of genome wide differences across the *H. erato* radiation, we sequenced the genomes of 101 individuals to 15-30x coverage, effectively genotyping an average of 62 % of the genome for each individual (Table S 10). Additionally, we sequenced the genomes of 15 individuals from 8 outgroup species to 15-30x coverage (Table S 11).

Table S 10. *Heliconius erato* sampling. If individuals are sampled near hybrid zones, the hybridizing race is indicated.

Sample ID	Genus	Species	Race	location	country	Hybrid zone	SRA accession
BC2115	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	Brokopondo	Suriname	<i>erato + hydara</i>	SAMN05224103
BC2124	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	Brokopondo	Suriname	<i>erato + hydara</i>	SAMN05224104
STRIWOM5779	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	Brokopondo	Suriname	<i>erato + hydara</i>	SAMN05224208
STRIWOM5780	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	Brokopondo	Suriname	<i>erato + hydara</i>	SAMN05224209
STRIWOM5781	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	Brokopondo	Suriname	<i>erato + hydara</i>	SAMN05224210
M3661	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224096
M3662	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224097
M3663	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224098
M3664	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224099
STRIWOM0057	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224192
STRIWOM0058	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224193
STRIWOM0059	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	Rio Calima	Colombia	-	SAMN05224194
cyrbia004	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	Balsas	Ecuador	-	SAMN05224122
cyrbia005	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	Balsas	Ecuador	-	SAMN05224123
cyrbia023	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	Balsas	Ecuador	-	SAMN05224124
cyrbia024	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	Balsas	Ecuador	-	SAMN05224125
PetED3	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Gamboa	Panama	<i>hydara + demophoon</i>	SAMN05224182
PetED4	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Gamboa	Panama	<i>hydara + demophoon</i>	SAMN05224183
PetED5	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Gamboa	Panama	<i>hydara + demophoon</i>	SAMN05224184
PetED6	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Gamboa	Panama	<i>hydara + demophoon</i>	SAMN05224185
STRIWOM0033	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224188

STRIWOM0082	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224195
STRIWOM0087	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224196
STRIWOM1284	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224198
STRIWOM5353	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224202
STRIWOM5362	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224203
GS020redo	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224127
GS021redo	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224128
NCS1671	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224154
NCS1672	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224155
NCS1673	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224156
NCS1674	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224157
NCS1675	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	San Cristobal	Peru	<i>favorinus + emma</i>	SAMN05224158
NCS2005	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224160
NCS2012	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224161
NCS2020	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224162
NCS2023	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224163
NCS2025	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224164
NCS2556	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224174
BC3277	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224110
BC3278	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224111
BC3280	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224112
BC3281	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224113
BC3282	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224114
GS012redo	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	,Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224126
NCS0471	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224148
NCS0473	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224149
NCS0476	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224150
NCS0478	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224151
NCS0479	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224152
NCS2554	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224172
NCS2555	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	Urahuasha	Peru	<i>favorinus + emma</i>	SAMN05224173
himera001	<i>Heliconius</i>	<i>himera</i>	-	Vilcabamba	Ecuador	-	SAMN05224132

himera002	<i>Heliconius</i>	<i>himera</i>	-	Vilcabamba	Ecuador	-	SAMN05224133
himera003	<i>Heliconius</i>	<i>himera</i>	-	Vilcabamba	Ecuador	-	SAMN05224134
himera006	<i>Heliconius</i>	<i>himera</i>	-	Vilcabamba	Ecuador	-	SAMN05224135
himera030	<i>Heliconius</i>	<i>himera</i>	-	Vilcabamba	Ecuador	-	SAMN05224136
NCS1179	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224153
NCS1979	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224159
NCS2080	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224165
NCS2211	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224166
NCS2217	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224167
NCS2574	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224175
NCS2581	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224176
NCS2609	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Kaw	French Guiana	<i>hydara + erato</i>	SAMN05224177
STRIWOM0039	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224189
STRIWOM0040	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224190
STRIWOM0088	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224197
STRIWOM5193	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224200
STRIWOM5351	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224201
STRIWOM0042	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	Lago Bayano	Panama	<i>hydara + demophoon</i>	SAMN05224191
BC0411	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	Ahuano	Ecuador	<i>notabilis + lativitta</i>	SAMN05224101
lativitta01	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	Ahuano	Ecuador	<i>notabilis + lativitta</i>	SAMN05224137
lativitta02	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	Ahuano	Ecuador	<i>notabilis + lativitta</i>	SAMN05224138
lativitta03	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	Ahuano	Ecuador	<i>notabilis + lativitta</i>	SAMN05224139
lativitta04	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	Jondachi	Ecuador	<i>notabilis + lativitta</i>	SAMN05224140
BC3223	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224105
BC3224	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224106
BC3225	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224107
BC3227	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224108
BC3228	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Huamboya	Ecuador	<i>notabilis + etylus</i>	SAMN05224109
BC0410	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Mera	Ecuador	<i>notabilis + lativitta</i>	SAMN05224100
notabilis01	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Mera	Ecuador	<i>notabilis + lativitta</i>	SAMN05224178
notabilis02	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Mera	Ecuador	<i>notabilis + lativitta</i>	SAMN05224179
notabilis03	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Mera	Ecuador	<i>notabilis + lativitta</i>	SAMN05224180

notabilis04	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	Mera	Ecuador	<i>notabilis + lativitta</i>	SAMN05224181
CA51	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	Campeche	Mexico	-	SAMN05224115
CA53	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	Campeche	Mexico	-	SAMN05224116
CA54	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	Campeche	Mexico	-	SAMN05224117
CA55	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	Campeche	Mexico	-	SAMN05224118
CA56	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	Campeche	Mexico	-	SAMN05224119
MRK_MX09_01	<i>Heliconius</i>	<i>erato</i>	<i>cruentus</i>	Puerto Vallarta	Mexico	-	SAMN05224147
5732	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	Samaipata	Bolivia	-	SAMN05224204
5742	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	Samaipata	Bolivia	-	SAMN05224205
5765	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	Samaipata	Bolivia	-	SAMN05224206
5766	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	Samaipata	Bolivia	-	SAMN05224207
M3654	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	Queremal	Colombia	-	SAMN05224141
M3655	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	Queremal	Colombia	-	SAMN05224142
M3656	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	Queremal	Colombia	-	SAMN05224143
M3657	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	Queremal	Colombia	-	SAMN05224144
M3659	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	Queremal	Colombia	-	SAMN05224145

Table S 11. Outgroup sampling.

Sample ID	Genus	Species	location	country	Hybrid Zone	SRA accession
char001	<i>Heliconius</i>	<i>charithonia</i>	Cambalache, Yauco	Puerto Rico	-	SAMN05224120
char002	<i>Heliconius</i>	<i>charithonia</i>	Cambalache, Yauco	Puerto Rico	-	SAMN05224121
NCS2511	<i>Heliconius</i>	<i>clysonimus</i>	Mina Negra	Peru	-	SAMN05224168
NCS2512	<i>Heliconius</i>	<i>clysonimus</i>	Mina Negra	Peru	-	SAMN05224169
STRIWOM2312	<i>Heliconius</i>	<i>hecalesia</i>	Boquete	Panama	-	SAMN05224199
LMCI94-13	<i>Heliconius</i>	<i>hermathena</i>	Santarém, Pará	Brazil	-	SAMN05224129
LMCI94-14	<i>Heliconius</i>	<i>hermathena</i>	Santarém, Pará	Brazil	-	SAMN05224130
LMCI94-15	<i>Heliconius</i>	<i>hermathena</i>	Santarém, Pará	Brazil	-	SAMN05224131
MRKhort2	<i>Heliconius</i>	<i>hortense</i>	Buttterfly Farm	El Salvador	-	SAMN05224146
STRIWOM5792	<i>Heliconius</i>	<i>ricini</i>	Brokopondo	Suriname	-	SAMN05224211
BC2004	<i>Heliconius</i>	<i>sara</i>	Brokopondo	Suriname	-	SAMN05224102
MRK_EC_08_025	<i>Heliconius</i>	<i>sara</i>	Mindo	Ecuador	-	SAMN05224186
sar08025	<i>Heliconius</i>	<i>sara</i>	Unknown	Unknown	-	SAMN04412545
NCS2541	<i>Heliconius</i>	<i>telesiphe</i>	Puente Chalayaco	Peru	-	SAMN05224170
NCS2550	<i>Heliconius</i>	<i>telesiphe</i>	Mina Negra	Peru	-	SAMN05224171

Table S 12. *Heliconius erato* mapping and genotyping results.

Sample ID	Genus	Species	Race	Reads	Mapped (%)	Properly paired (%)	Genotyped (%)	Homozygous (%)	Heterozygous (%)
BC2115	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	124725685	92.98	66.70	63.54	96.75	3.25
BC2124	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	134483280	93.03	66.66	64.32	96.73	3.27
STRIWOM5779	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	146432487	95.04	73.42	65.14	96.75	3.25
STRIWOM5780	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	121593810	94.40	71.28	62.69	96.89	3.11
STRIWOM5781	<i>Heliconius</i>	<i>erato</i>	<i>amalfreda</i>	108858237	94.82	72.88	60.90	96.90	3.10
M3661	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	99124314	96.36	70.01	65.48	98.83	1.17
M3662	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	131202069	94.61	69.20	67.16	98.97	1.03
M3663	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	124765688	96.60	75.24	67.00	98.97	1.03
M3664	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	158840593	97.28	74.82	68.42	98.89	1.11
STRIWOM0057	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	115608137	94.16	74.69	60.63	98.99	1.01
STRIWOM0058	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	115958053	95.81	75.97	60.70	98.97	1.03
STRIWOM0059	<i>Heliconius</i>	<i>erato</i>	<i>chestertonii</i>	135669920	96.03	76.76	65.17	99.00	1.00
cyrbia004	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	118648654	95.50	76.08	61.46	97.77	2.23
cyrbia005	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	108428572	95.55	76.07	60.08	97.89	2.11
cyrbia023	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	107602073	95.33	75.84	63.66	97.84	2.16
cyrbia024	<i>Heliconius</i>	<i>erato</i>	<i>cyrbia</i>	106407244	95.75	76.70	59.91	97.85	2.15
PetED3	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	147571378	97.61	84.17	65.27	97.08	2.92
PetED4	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	147831384	96.32	83.27	64.26	97.05	2.95
PetED5	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	124430446	97.27	83.64	60.84	97.09	2.91
PetED6	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	147157885	97.66	84.25	64.75	97.06	2.94
STRIWOM0033	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	103265835	96.40	78.96	61.15	97.01	2.99
STRIWOM0082	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	132459907	94.90	74.73	61.73	96.85	3.15
STRIWOM0087	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	254747400	95.40	73.43	67.96	96.97	3.03
STRIWOM1284	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	144143162	94.62	71.57	70.29	97.18	2.82
STRIWOM5353	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	137933040	94.75	73.14	66.47	96.95	3.05
STRIWOM5362	<i>Heliconius</i>	<i>erato</i>	<i>demophoon</i>	132459907	94.90	74.73	65.53	97.00	3.00
GS020redo	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	127435592	94.02	75.37	56.28	96.84	3.16
GS021redo	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	110321776	92.25	73.90	53.05	97.36	2.64
NCS1671	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	138212409	94.87	74.80	63.79	96.69	3.31

NCS1672	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	124260648	95.30	74.38	61.98	96.72	3.28
NCS1673	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	134588320	94.75	74.33	63.06	96.70	3.30
NCS1674	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	123541303	94.85	73.93	62.17	96.69	3.31
NCS1675	<i>Heliconius</i>	<i>erato</i>	<i>emma</i>	88975238	95.26	75.48	51.91	96.84	3.16
NCS2005	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	131970196	94.20	74.50	61.65	96.85	3.15
NCS2012	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	149713935	92.69	74.01	61.89	96.92	3.08
NCS2020	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	128119208	94.95	75.68	61.73	96.86	3.14
NCS2023	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	157127558	92.47	74.64	64.40	96.82	3.18
NCS2025	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	131170024	94.16	75.42	61.59	96.86	3.14
NCS2556	<i>Heliconius</i>	<i>erato</i>	<i>erato</i>	104241150	94.18	74.39	60.36	96.84	3.16
BC3277	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	86701039	94.39	67.40	57.11	96.76	3.24
BC3278	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	98511471	94.63	67.34	58.65	96.80	3.20
BC3280	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	89246745	93.49	66.23	55.80	96.78	3.22
BC3281	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	102130480	94.55	68.05	61.00	96.71	3.29
BC3282	<i>Heliconius</i>	<i>erato</i>	<i>etylus</i>	103368694	94.50	67.47	59.72	96.78	3.22
GS012redo	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	140981241	94.98	76.95	61.22	96.87	3.13
NCS0471	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	107620175	93.25	73.57	60.09	96.83	3.17
NCS0473	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	101638593	94.31	74.54	58.62	96.84	3.16
NCS0476	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	122366761	93.19	73.12	65.12	96.76	3.24
NCS0478	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	144088132	95.29	73.92	65.90	96.82	3.18
NCS0479	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	116707167	93.72	72.92	64.61	96.74	3.26
NCS2554	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	104925650	94.64	74.75	62.35	97.13	2.87
NCS2555	<i>Heliconius</i>	<i>erato</i>	<i>favorinus</i>	135257331	95.51	75.92	66.13	97.31	2.69
himera001	<i>Heliconius</i>	<i>himera</i>		98013649	95.55	75.60	59.50	98.70	1.30
himera002	<i>Heliconius</i>	<i>himera</i>		106525064	95.19	75.01	61.14	98.69	1.31
himera003	<i>Heliconius</i>	<i>himera</i>		110558877	95.47	75.20	61.51	98.64	1.36
himera006	<i>Heliconius</i>	<i>himera</i>		79235786	94.77	74.00	53.26	98.73	1.27
himera030	<i>Heliconius</i>	<i>himera</i>		75307798	95.00	74.46	52.29	98.77	1.23
NCS1179	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	96419175	95.25	75.09	57.84	96.94	3.06
NCS1979	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	110070137	95.22	74.91	60.46	96.87	3.13
NCS2080	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	107728439	95.79	75.31	59.48	96.98	3.02
NCS2211	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	126470247	94.52	74.48	63.23	96.85	3.15

NCS2217	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	142163032	95.26	74.81	65.09	96.83	3.17
NCS2574	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	61179802	94.03	74.34	42.84	98.16	1.84
NCS2581	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	141887122	95.44	76.47	63.43	97.03	2.97
NCS2609	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	147479030	95.86	76.74	63.30	97.19	2.81
STRIWOM0039	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	109671810	96.47	78.71	61.63	97.00	3.00
STRIWOM0040	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	112212395	96.01	78.33	62.74	96.98	3.02
STRIWOM0088	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	56427236	94.78	71.99	68.49	96.77	3.23
STRIWOM5193	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	159885870	95.32	72.08	65.96	96.79	3.21
STRIWOM5351	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	247932516	95.42	71.66	63.25	96.79	3.21
STRIWOM0042	<i>Heliconius</i>	<i>erato</i>	<i>hydara</i>	113978486	95.56	77.63	60.65	97.05	2.95
BC0411	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	167711422	90.91	72.37	64.95	96.82	3.18
lativitta01	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	112167739	94.35	75.00	58.21	98.01	1.99
lativitta02	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	143079745	94.85	75.78	61.23	96.97	3.03
lativitta03	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	163526599	95.72	76.15	63.04	96.84	3.16
lativitta04	<i>Heliconius</i>	<i>erato</i>	<i>lativitta</i>	171477845	93.92	74.37	63.87	96.91	3.09
BC0410	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	160272309	95.12	76.27	65.52	96.81	3.19
BC3223	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	89779257	94.47	67.80	57.70	96.73	3.27
BC3224	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	91802518	94.41	67.99	57.13	96.78	3.22
BC3225	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	96513836	94.51	67.81	57.97	96.77	3.23
BC3227	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	91732038	93.23	66.62	56.51	96.86	3.14
BC3228	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	76146914	94.39	68.31	51.89	96.81	3.19
notabilis01	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	115174166	94.54	74.33	59.26	96.90	3.10
notabilis02	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	120120673	92.65	72.79	60.73	96.85	3.15
notabilis03	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	132385897	93.92	73.73	62.06	96.96	3.04
notabilis04	<i>Heliconius</i>	<i>erato</i>	<i>notabilis</i>	122086027	95.33	74.95	61.54	96.92	3.08
CA51	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	84636118	96.48	75.30	63.25	98.68	1.32
CA53	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	100757305	95.01	73.41	65.97	98.65	1.35
CA54	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	127961432	95.31	74.76	67.80	98.63	1.37
CA55	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	97940026	96.32	75.62	64.96	98.67	1.33
CA56	<i>Heliconius</i>	<i>erato</i>	<i>petiverana</i>	114976134	96.09	74.38	67.12	98.65	1.35
MRK_MX09_01	<i>Heliconius</i>	<i>erato</i>	<i>cruentus</i>	133056919	93.89	68.99	64.44	99.35	0.65
5732	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	113500563	94.79	68.00	60.68	96.64	3.36

5742	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	134943613	92.91	69.17	63.59	96.63	3.37
5765	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	130522327	95.32	72.98	63.21	96.64	3.36
5766	<i>Heliconius</i>	<i>erato</i>	<i>phyllis</i>	93584774	95.13	67.84	61.26	96.53	3.47
M3654	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	128044805	94.78	74.26	65.31	97.63	2.37
M3655	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	154784987	94.33	73.43	66.63	97.66	2.34
M3656	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	130613531	94.55	74.40	65.31	97.67	2.33
M3657	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	140573742	94.75	75.51	64.74	97.70	2.30
M3659	<i>Heliconius</i>	<i>erato</i>	<i>venus</i>	142004452	94.02	73.57	66.27	98.12	1.88

Table S 13. Outgroup mapping and genotyping results.

Sample ID	Genus	Species	Reads	Mapped (%)	Properly paired (%)	Genotyped (%)	Homozygous (%)	Heterozygous (%)
char001	<i>Heliconius</i>	<i>charithonia</i>	140245309	77.91	47.78	40.45	99.53	0.47
char002	<i>Heliconius</i>	<i>charithonia</i>	79721501	79.07	44.26	30.40	98.91	1.09
NCS2511	<i>Heliconius</i>	<i>clysonimus</i>	92849122	88.31	60.67	39.26	98.81	1.19
NCS2512	<i>Heliconius</i>	<i>clysonimus</i>	112450950	89.29	62.08	41.37	98.78	1.22
STRIWOM2312	<i>Heliconius</i>	<i>hecalesia</i>	152544131	89.95	62.79	49.41	98.90	1.10
LMCI94-13	<i>Heliconius</i>	<i>hermathena</i>	145055665	89.08	61.96	55.52	99.77	0.23
LMCI94-14	<i>Heliconius</i>	<i>hermathena</i>	173637812	88.76	61.05	57.12	99.77	0.23
LMCI94-15	<i>Heliconius</i>	<i>hermathena</i>	209772280	88.98	62.84	57.77	99.76	0.24
MRKhort2	<i>Heliconius</i>	<i>hortense</i>	141576626	85.68	57.42	48.57	98.98	1.02
STRIWOM5792	<i>Heliconius</i>	<i>ricini</i>	238855233	79.26	52.89	41.20	99.47	0.53
BC2004	<i>Heliconius</i>	<i>sara</i>	133231891	80.00	49.41	40.83	98.61	1.39
MRK_EC_08_025	<i>Heliconius</i>	<i>sara</i>	135516002	81.31	51.76	39.99	98.85	1.15
sar08025	<i>Heliconius</i>	<i>sara</i>	70726663	79.63	46.34	32.59	98.90	1.10
NCS2541	<i>Heliconius</i>	<i>telesiphe</i>	110690751	89.07	61.13	42.14	98.94	1.06
NCS2550	<i>Heliconius</i>	<i>telesiphe</i>	122493430	88.96	59.24	44.65	98.89	1.11

S4.2. Whole genome phylogeny

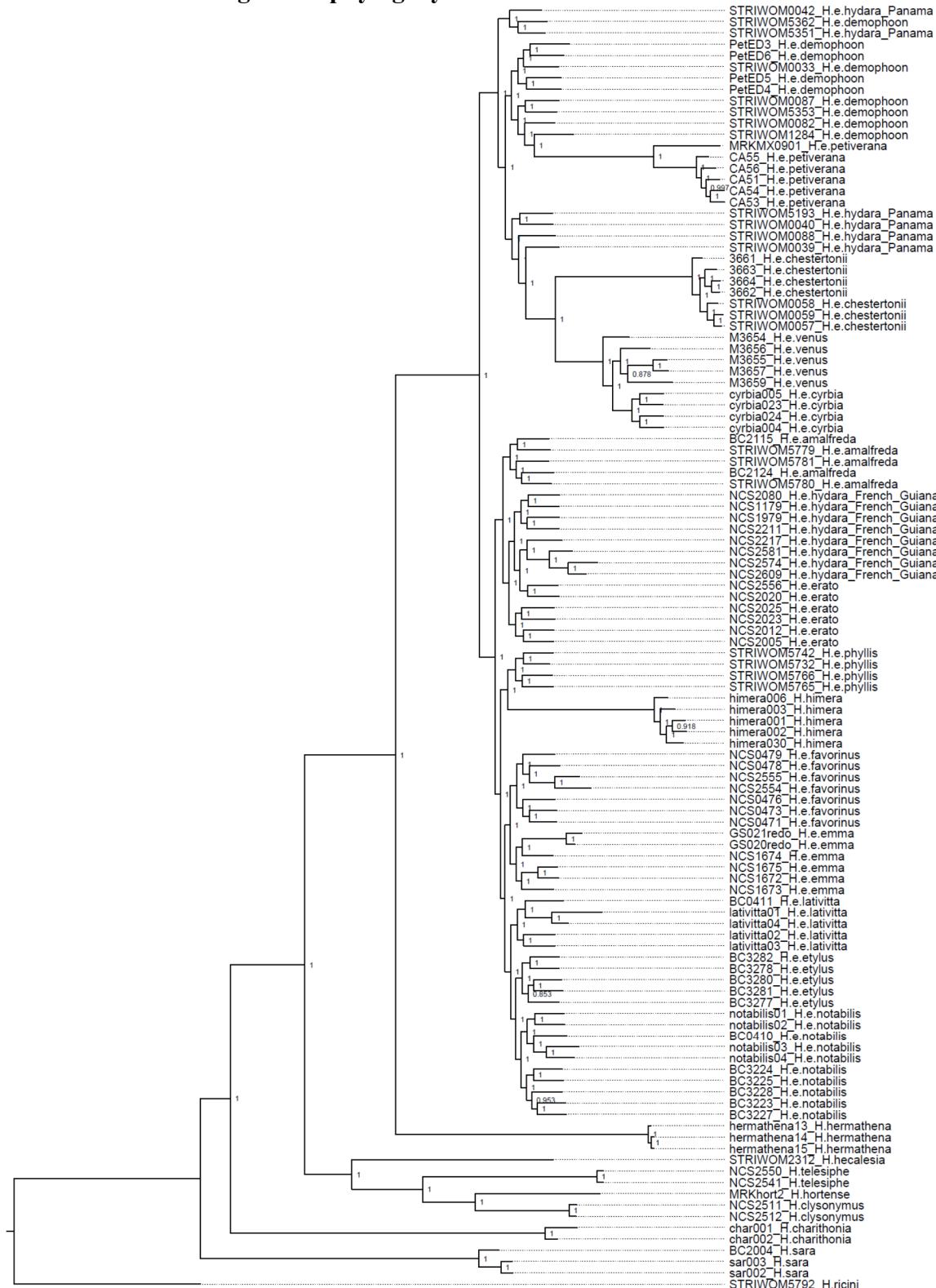


Figure S 5. Whole genome phylogeny. Tip labels represent sample ID and species. Node labels represent local support based on the Shimodaira-Hasegawa test.

S4.3. Whole genome population genetic statistics for hybridizing taxa

Genome wide comparisons of genomic divergence and diversity reveal narrow peaks only near pattern loci and a few other regions of the genome. Similar to F_{ST} , d_{XY} , an absolute measure of genetic divergence, revealed peaks near the color pattern loci (chromosomes 10, 13, 15 and 18), as well as on chromosomes 2 and 3, which are not known to house color pattern loci. On chromosome 2 a large region showed elevated divergence between rayed and postman races in Peru and the Guiana shield. The elevated divergence appears to expand nearly the entire chromosome, except the end regions, which likely results from structural differences that have prevented recombination and gene flow between the races. On chromosome 3, a narrow peak of divergence is consistently found between all hybridizing races, except the postman-like races in Panama (*H. e. demophoon* and *H. e. hydara*). This region also shows elevated levels of nucleotide diversity, which may be the result of incomplete sorting of ancestral variation or balancing selection. F_{ST} showed elevated levels of divergence on the Z chromosome relative to autosomes, however absolute divergence and nucleotide diversity were on average lower on the Z. Comparisons between *H. e. favorinus* and *H. e. emma* in Peru did reveal additional peaks of divergence that were not replicated in any other comparisons. Among all the hybridizing races, these two races in Peru are the only that differ at each major color pattern locus and hybridization has been shown to be associated with other environmental variables, such as rainfall⁹, which could contribute to the greater number of divergent peaks across their genomes. Importantly, the only regions of the genome in which we found fixed allelic differences were at the color pattern loci, between hybridizing races that were divergently colored.

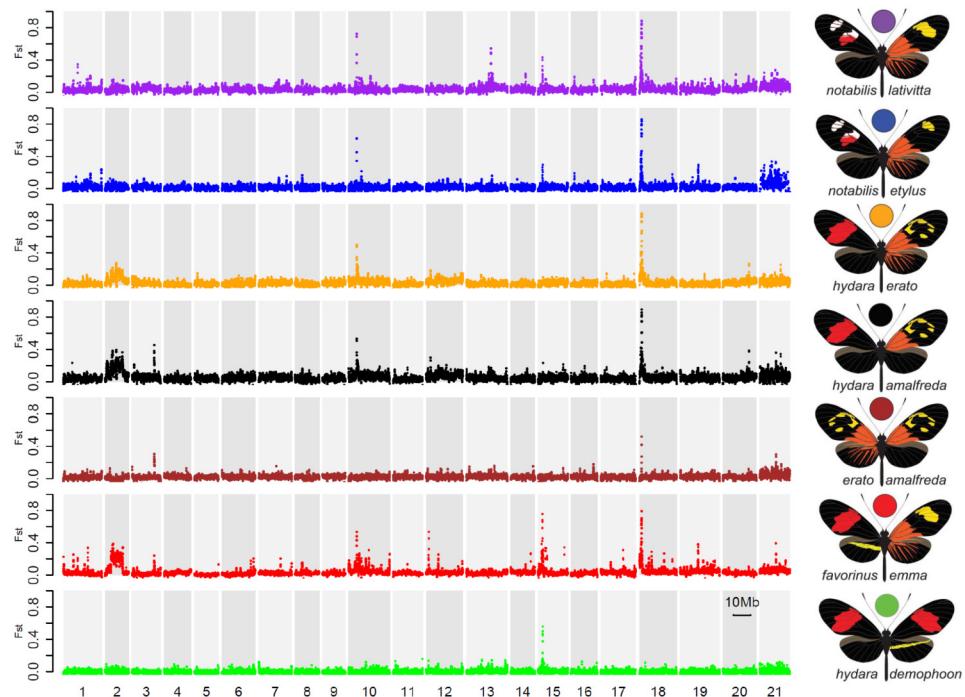


Figure S 6. F_{ST} across *Heliconius erato* phenotypic transition zones.

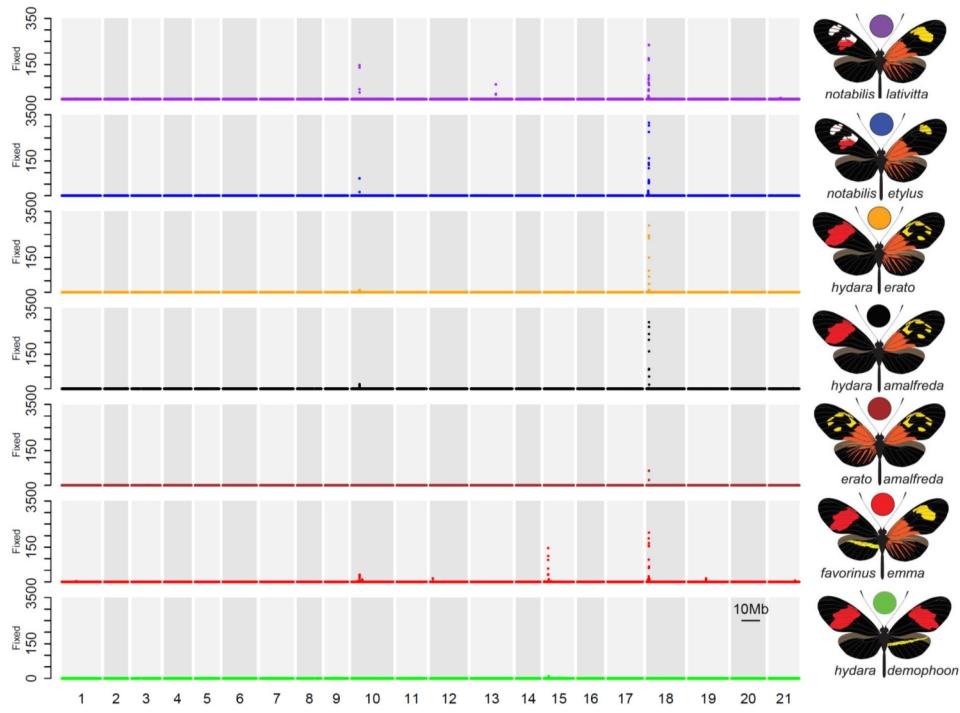


Figure S 7. Fixed differences across *Heliconius erato* phenotypic transition zones.

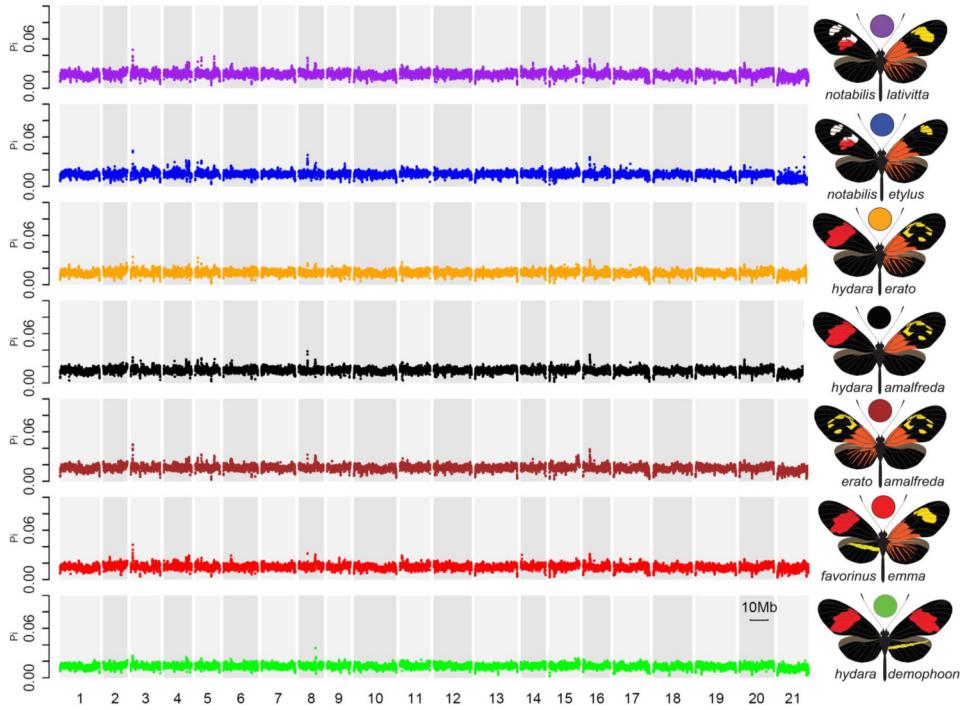


Figure S 8. Nucleotide diversity (Pi) in *Heliconius erato* phenotypic transition zones.

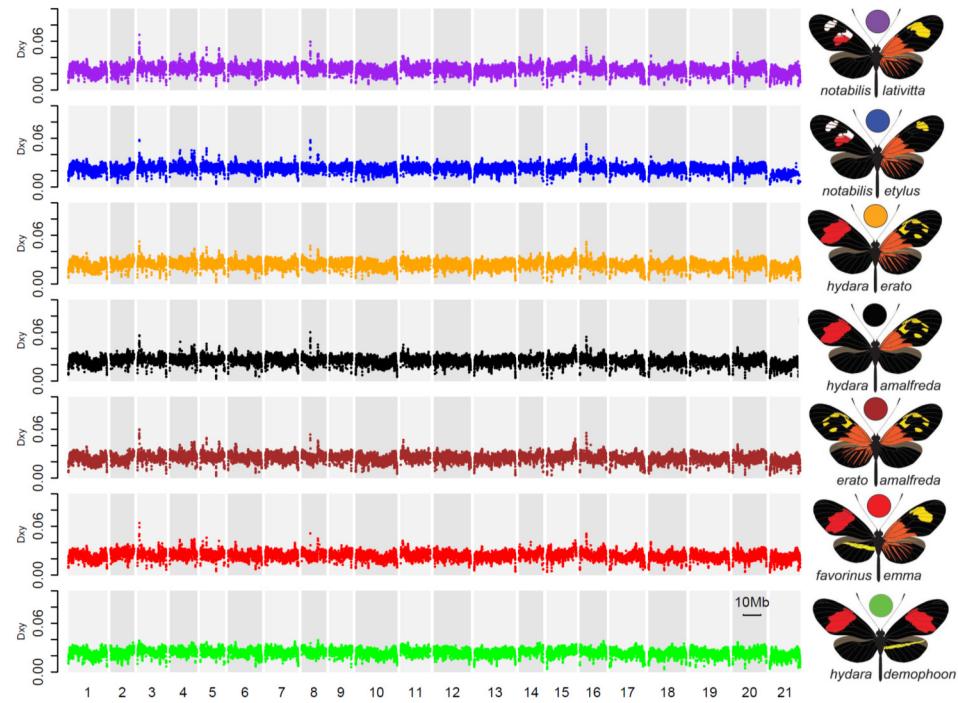


Figure S 9. Average pairwise differences (d_{XY}) across *Heliconius erato* phenotypic transition zones.

S5. Patterns at color pattern loci

Comparisons of hybridizing races that differ in wing color patterns proved to be extremely powerful for resolving putative regulatory regions of known color patterning genes. At each of the three major color pattern loci, not only were narrow peaks of divergence and genotype by phenotype associations identified between divergently colored races, but also these peaks were absent between hybridizing races that shared similar color pattern phenotypes. For example, between the hybridizing races *H. e. demophoon* and *H. e. hydara*, which do not differ in red pattern or forewing band shape, there were no peaks of divergence found near *optix* or *wntA*, as expected. Similarly, between the *H. e. hydara* and *H. e. erato/amalfreda*, none of which vary in yellow on the hindwing, there was no peak of divergence or fixed differences across the *Cr* locus (*cortex*). These negative results further support the potential functional roles in driving color pattern differences between the divergently colored hybridizing races.

S5.1. *wntA*

S5.1.1. Sliding window population statistics near *wntA*

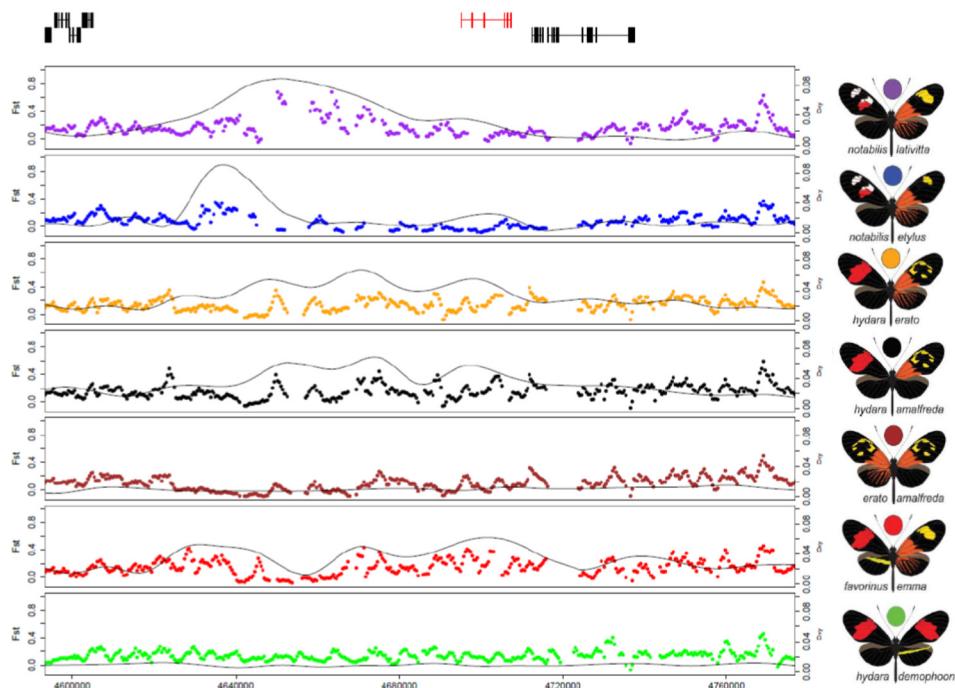


Figure S10. Sliding window F_{ST} (black line) and d_{XY} (points) near the *wntA* gene (red) on chromosome 10 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

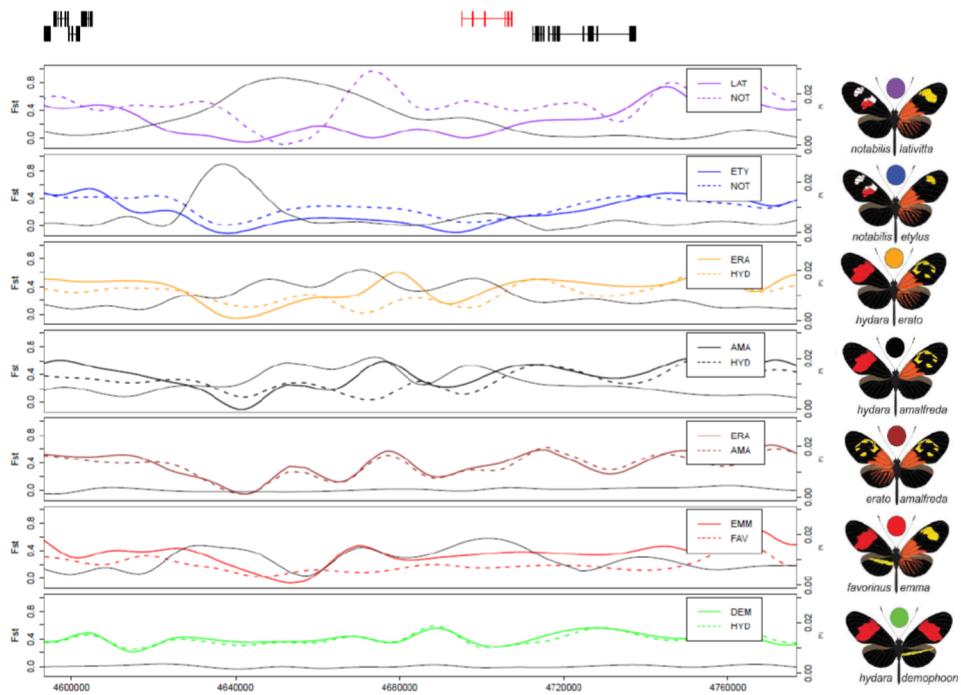


Figure S 11. Sliding window F_{ST} (black line) and nucleotide diversity (π) (colored lines) near the *wntA* gene (red) on chromosome 10 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

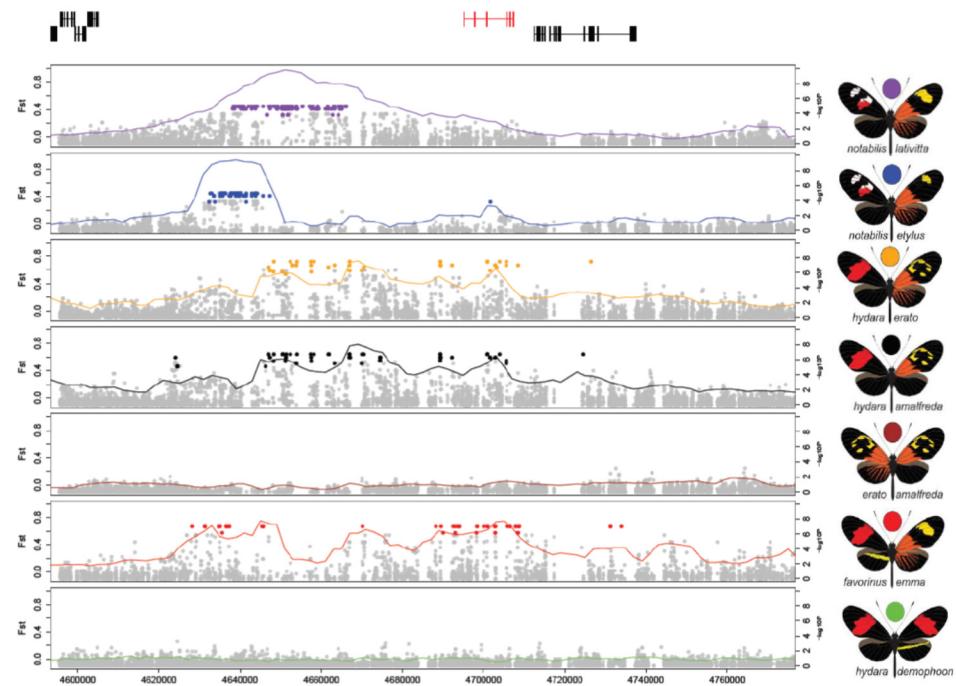


Figure S 12. F_{ST} (lines; 20 kb window, 5 kb step size) and association analysis (points) near the *wntA* gene on chromosome 10. Colored points represent associations estimated from fixed SNPs.

S5.1.2. Maximum likelihood trees for identified color pattern modules near *wntA*

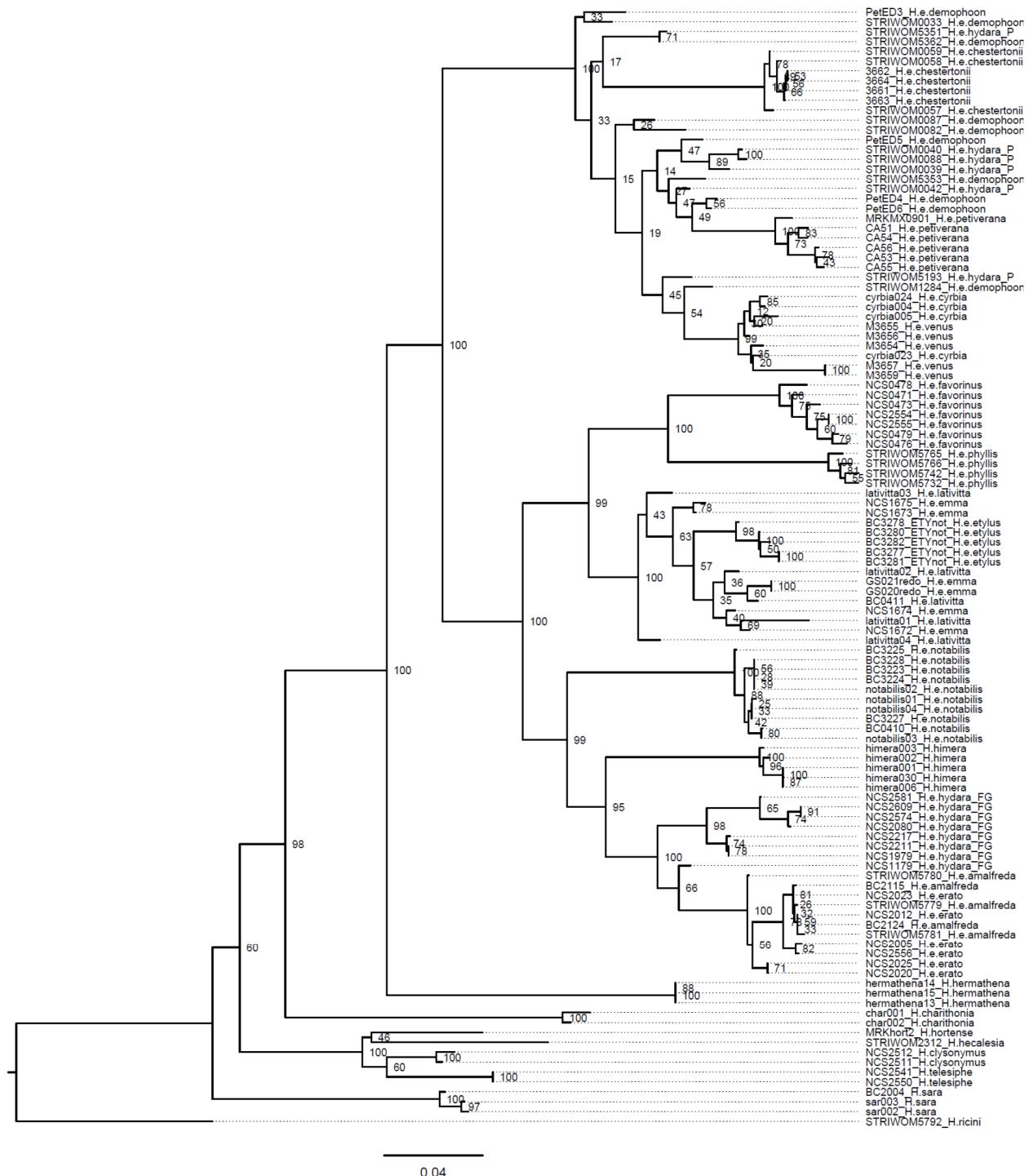


Figure S 13. Maximum likelihood tree for the *Sd* element near *wntA* on chromosome 10 (Herato1001 position 4624122-4647331) for all *H. erato* samples and closely related outgroup species.

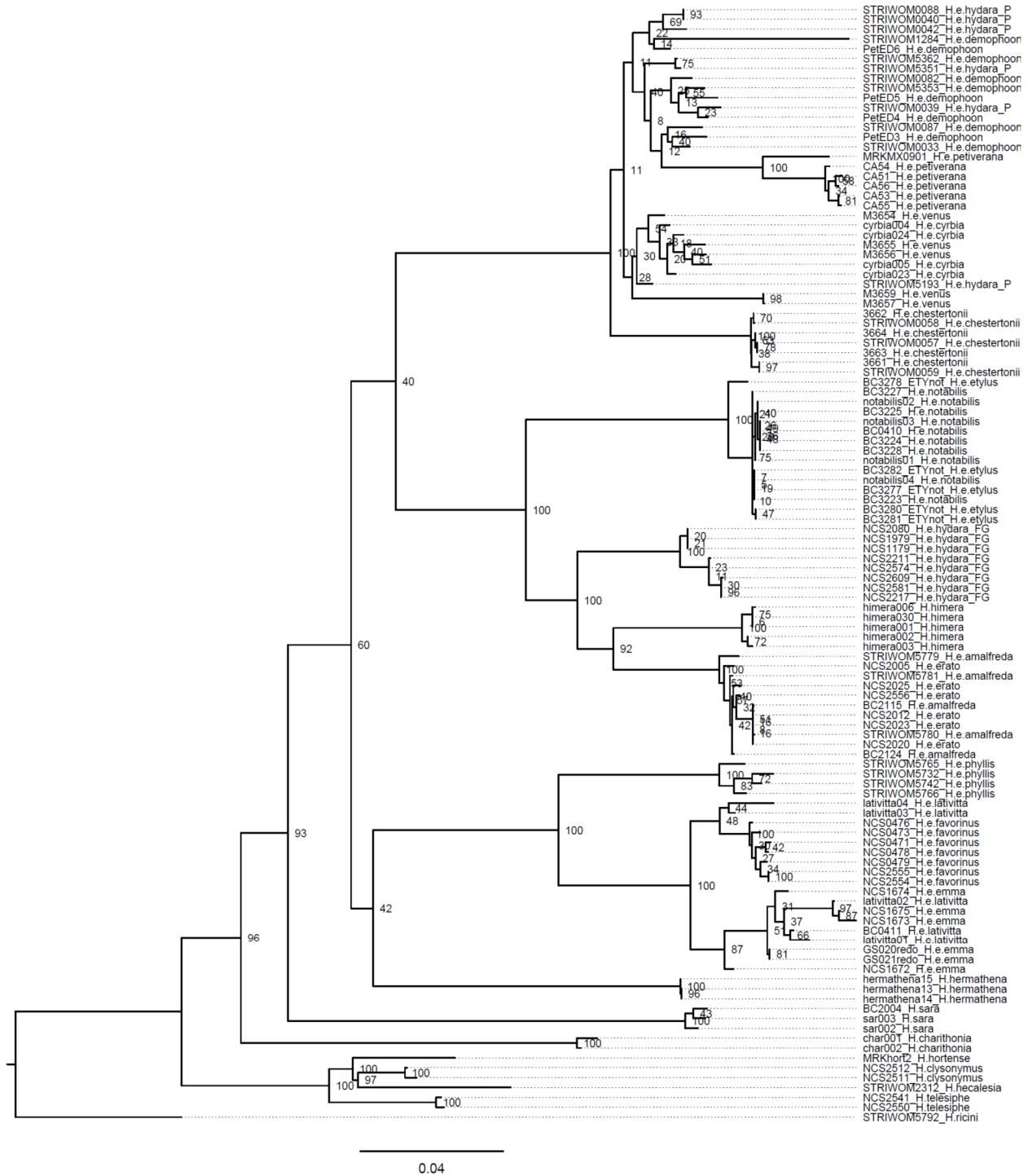


Figure S 14. Maximum likelihood tree for the *St* element near *wntA* on chromosome 10 (Herato1001 position 4647332-4674571) for all *H. erato* samples and closely related outgroup species.



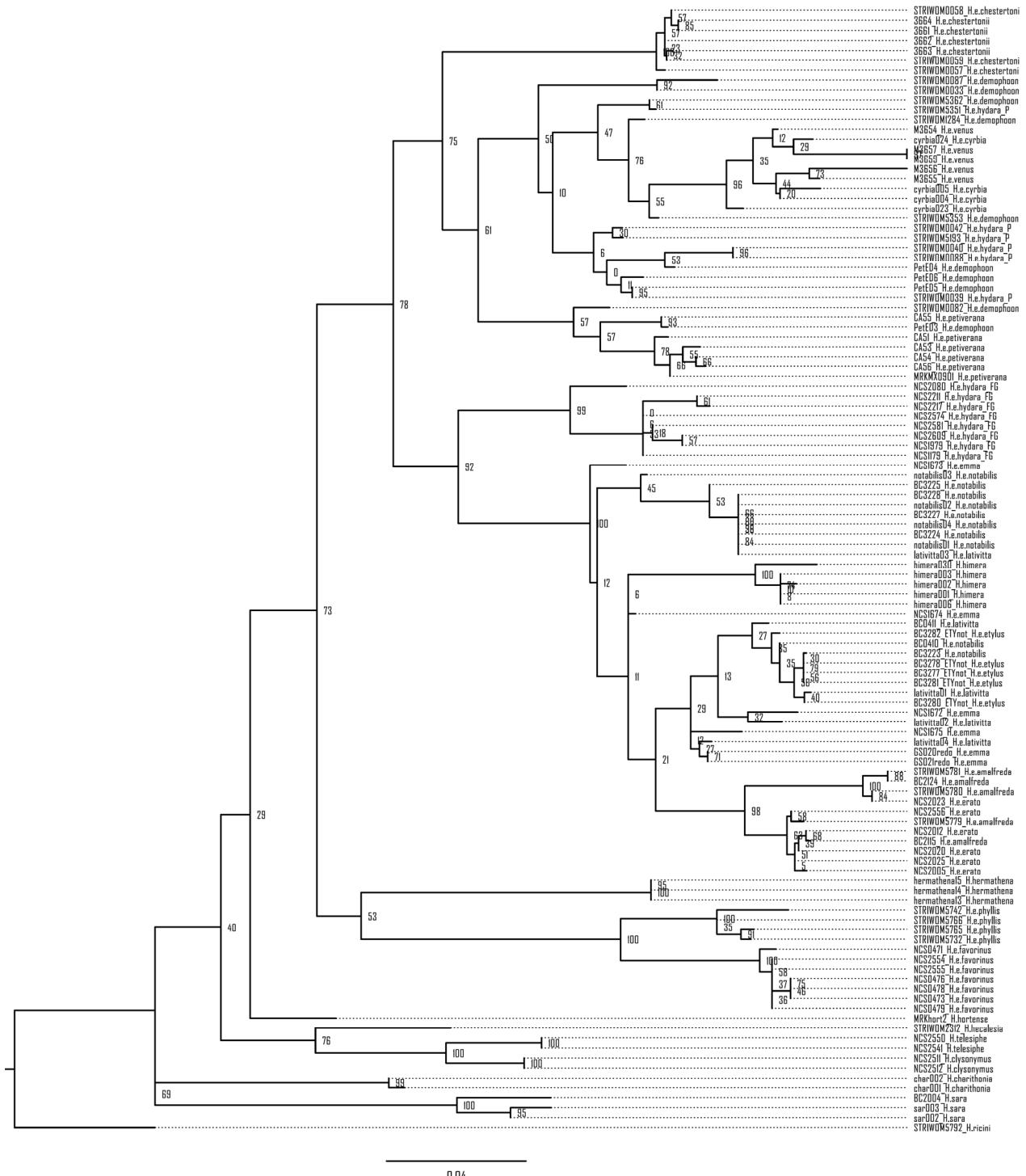


Figure S 16. Maximum likelihood tree for the *Ly2* element near *wntA* on chromosome 10 (Herato1001 position 4700932-4708441) for all *H. erato* samples and closely related outgroup species.

S5.2. Ro

S5.2.1. Sliding window population statistics near *Ro*

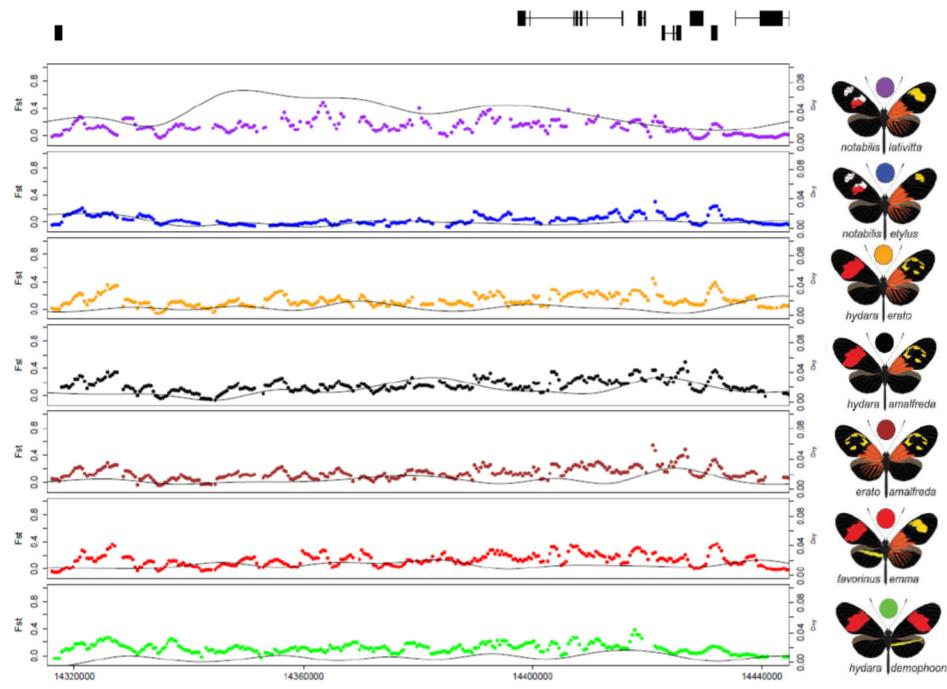


Figure S 17. Sliding window F_{ST} (black line) and d_{XY} (points) near the *Ro* locus on chromosome 13 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

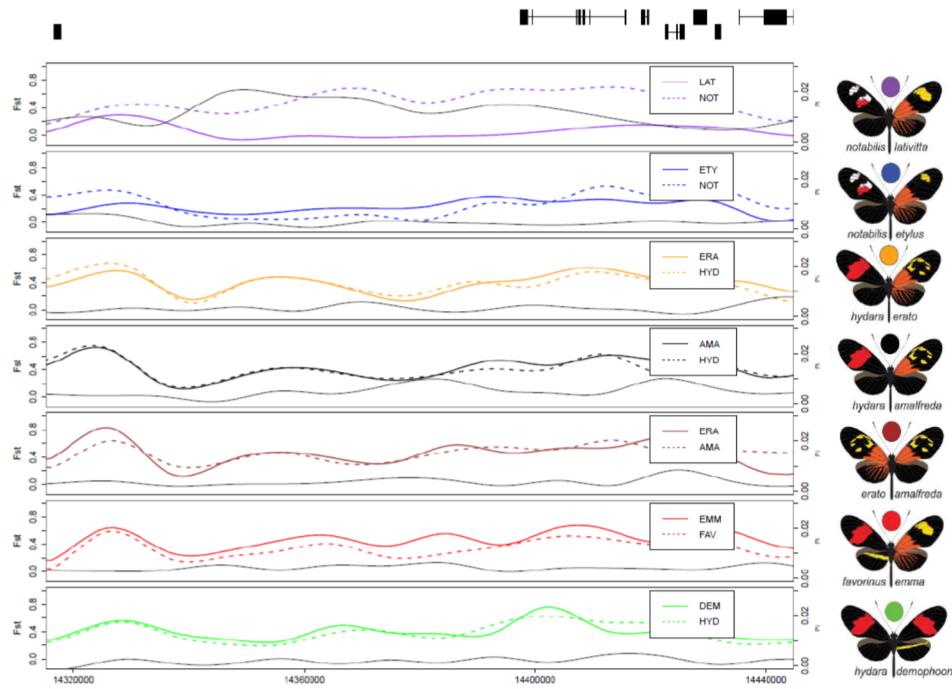


Figure S 18. Sliding window F_{ST} (black line) and nucleotide diversity (π) (colored lines) near the *Ro* locus on chromosome 13 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

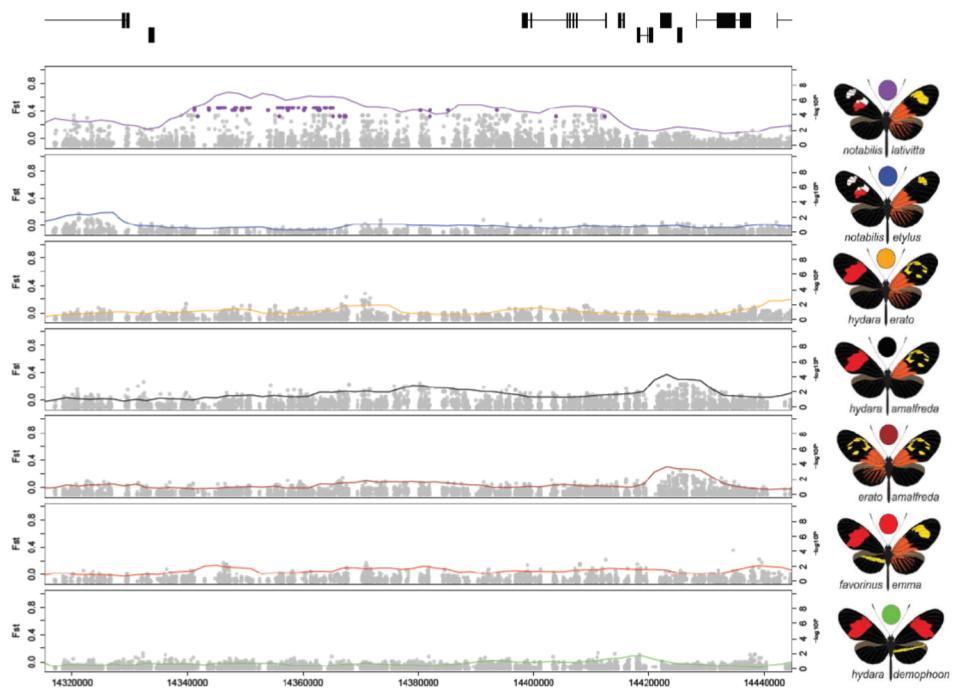


Figure S 19. F_{ST} (lines; 20 kb window, 5 kb step size) and association (points) analysis near the *Ro* locus on chromosome 13. Colored points represent associations estimated from fixed SNPs.

S5.2.2. Maximum likelihood tree for identified color pattern module near *Ro*

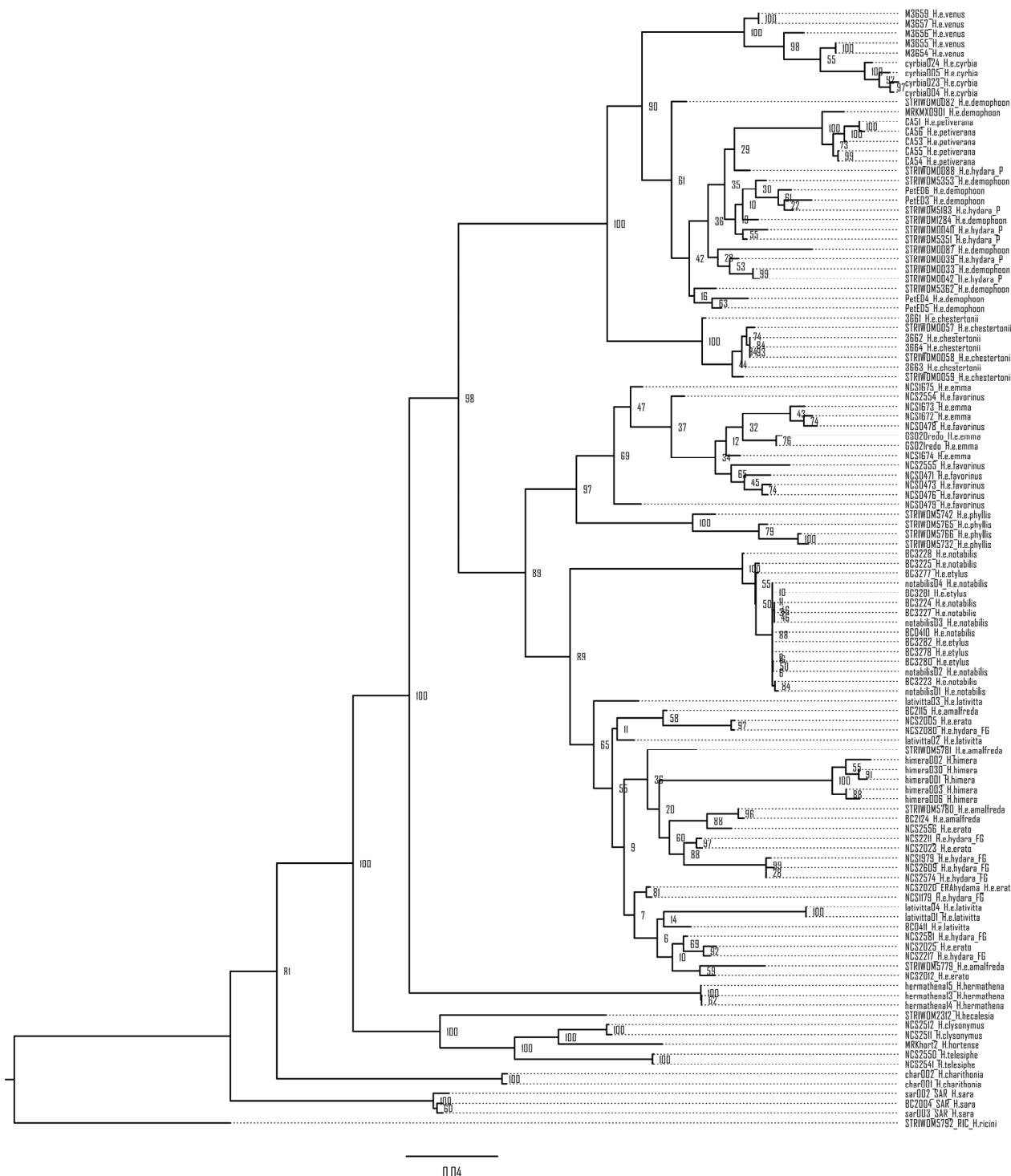


Figure S 20. Maximum likelihood tree for the *Ro* element on chromosome 13 (Herato1301 position 14341251-14367418) for all *H. erato* samples and closely related outgroup species.

S5.3. *Optix*

S5.3.1. Sliding window population statistics near *optix*

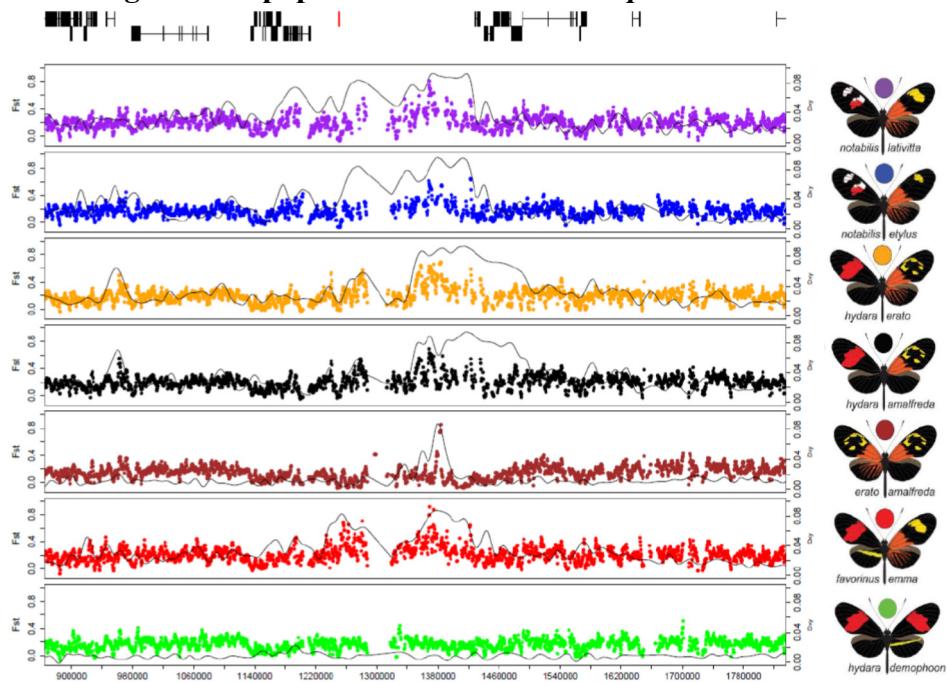


Figure S 21. Sliding window F_{ST} (black line) and d_{XY} (points) near the *optix* gene on chromosome 10 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

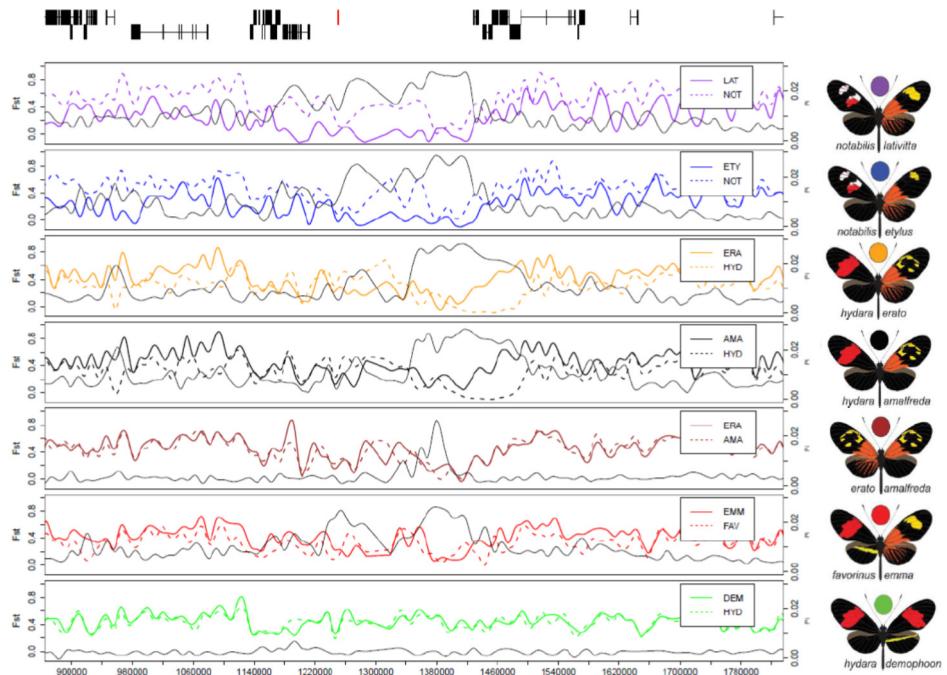


Figure S 22. Sliding window F_{ST} (black line) and nucleotide diversity (P_i) (colored lines) near the *optix* gene on chromosome 18 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

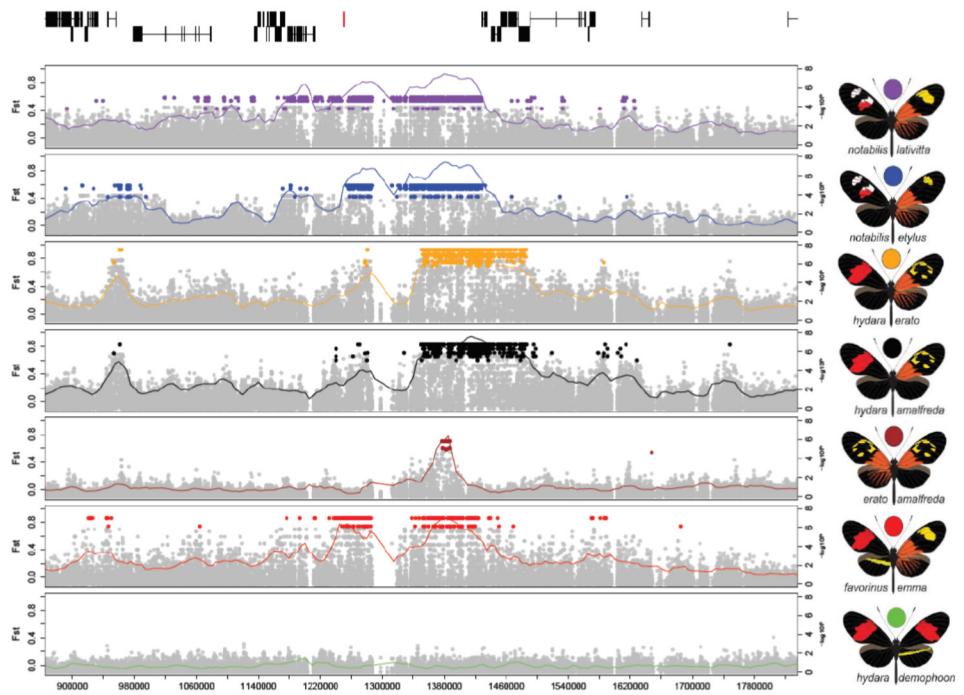


Figure S 23. F_{ST} (lines; 20 kb window, 5 kb step size) and association (points) analysis near the *optix* gene on chromosome 18. Colored points represent associations estimated from fixed SNPs.

S5.3.2. Maximum likelihood trees for identified color pattern modules near *optix*

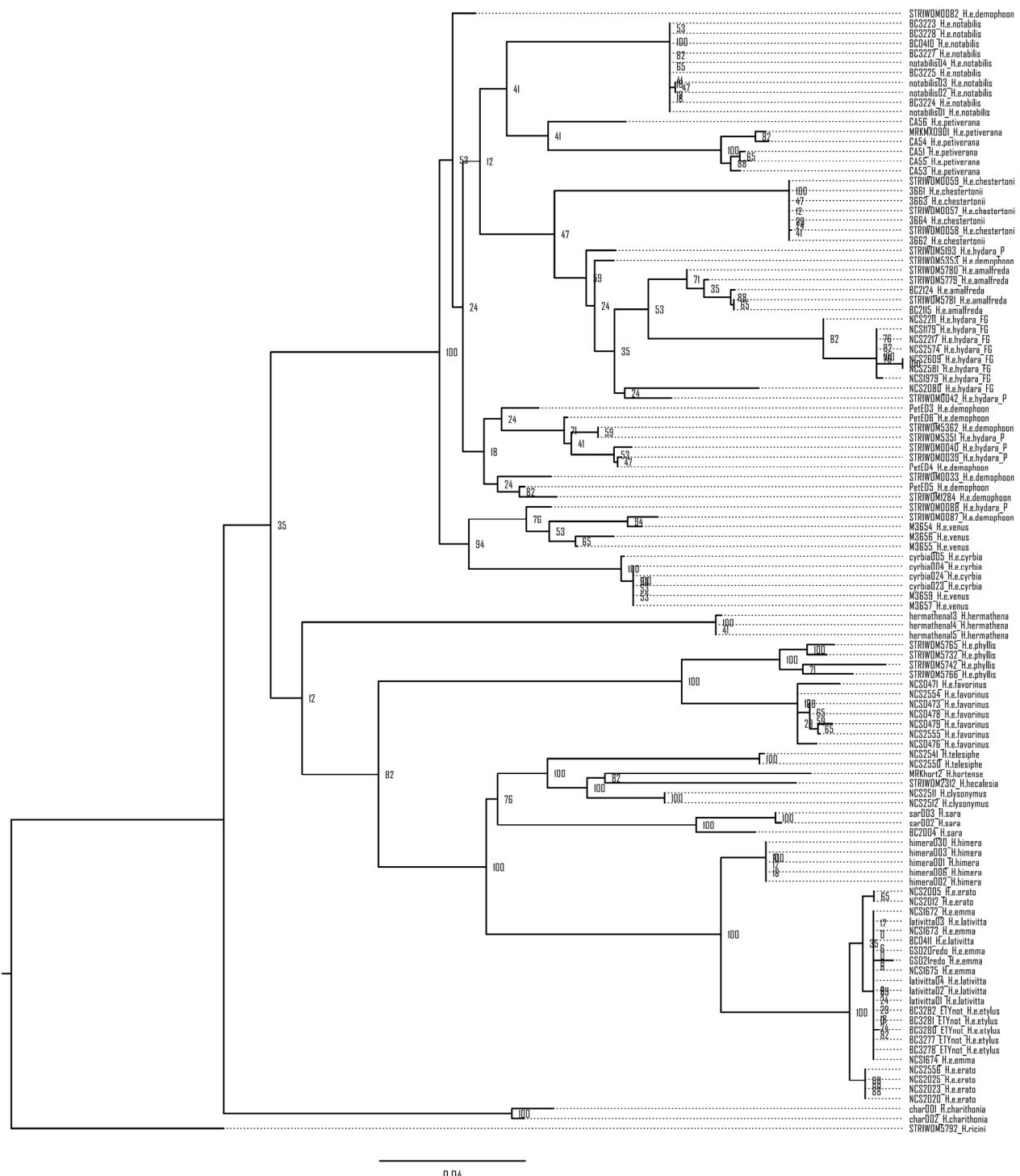


Figure S 24. Maximum likelihood tree for the *R* element near *optix* on chromosome 18 (Herato1801 position 1377801-1384841) for all *H. erato* samples and closely related outgroup species.

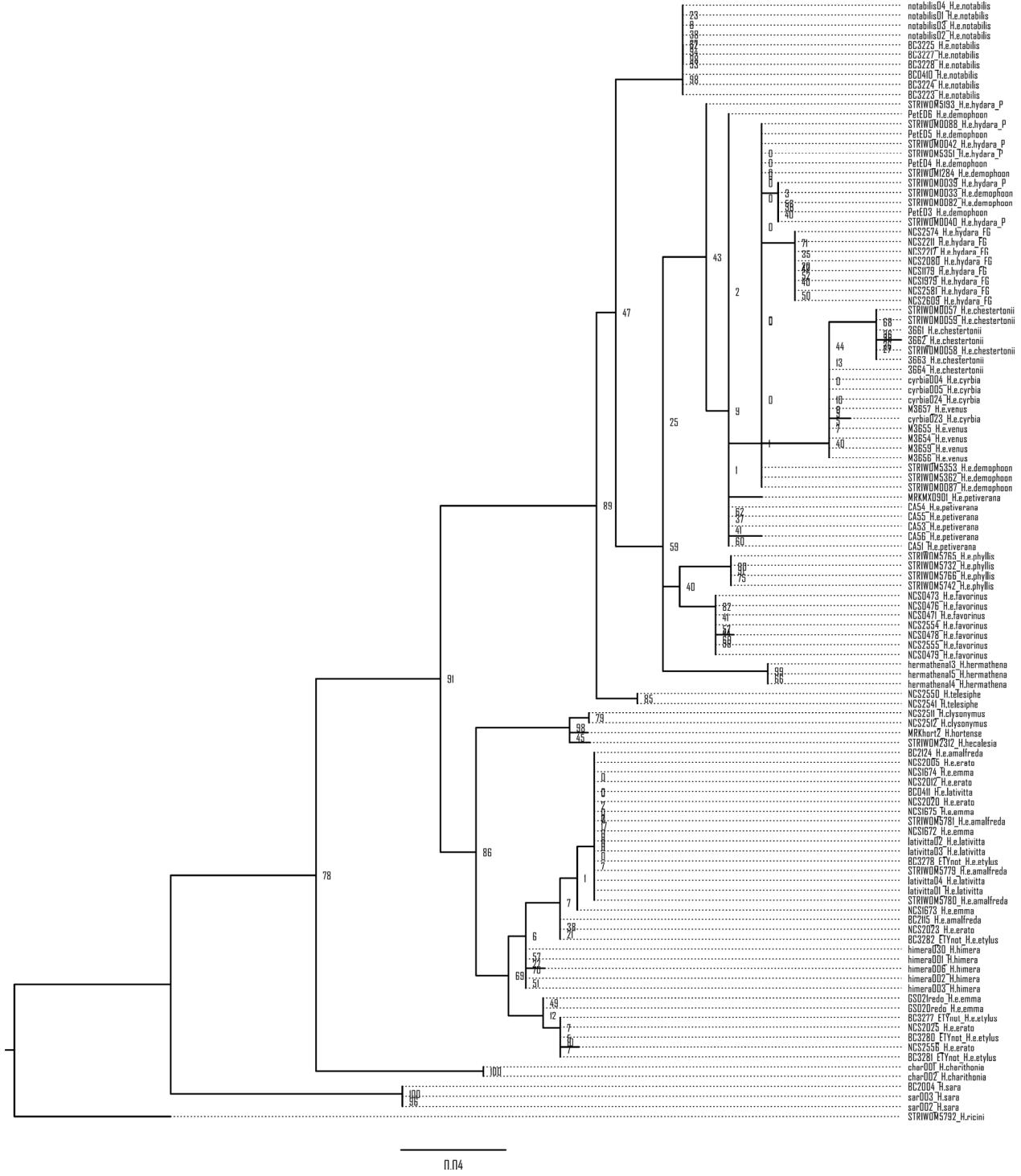


Figure S 25. Maximum likelihood tree for the *Y1* element near *optix* on chromosome 18 (Herato1801 position 1403328-1403791) for all *H. erato* samples and closely related outgroup species.

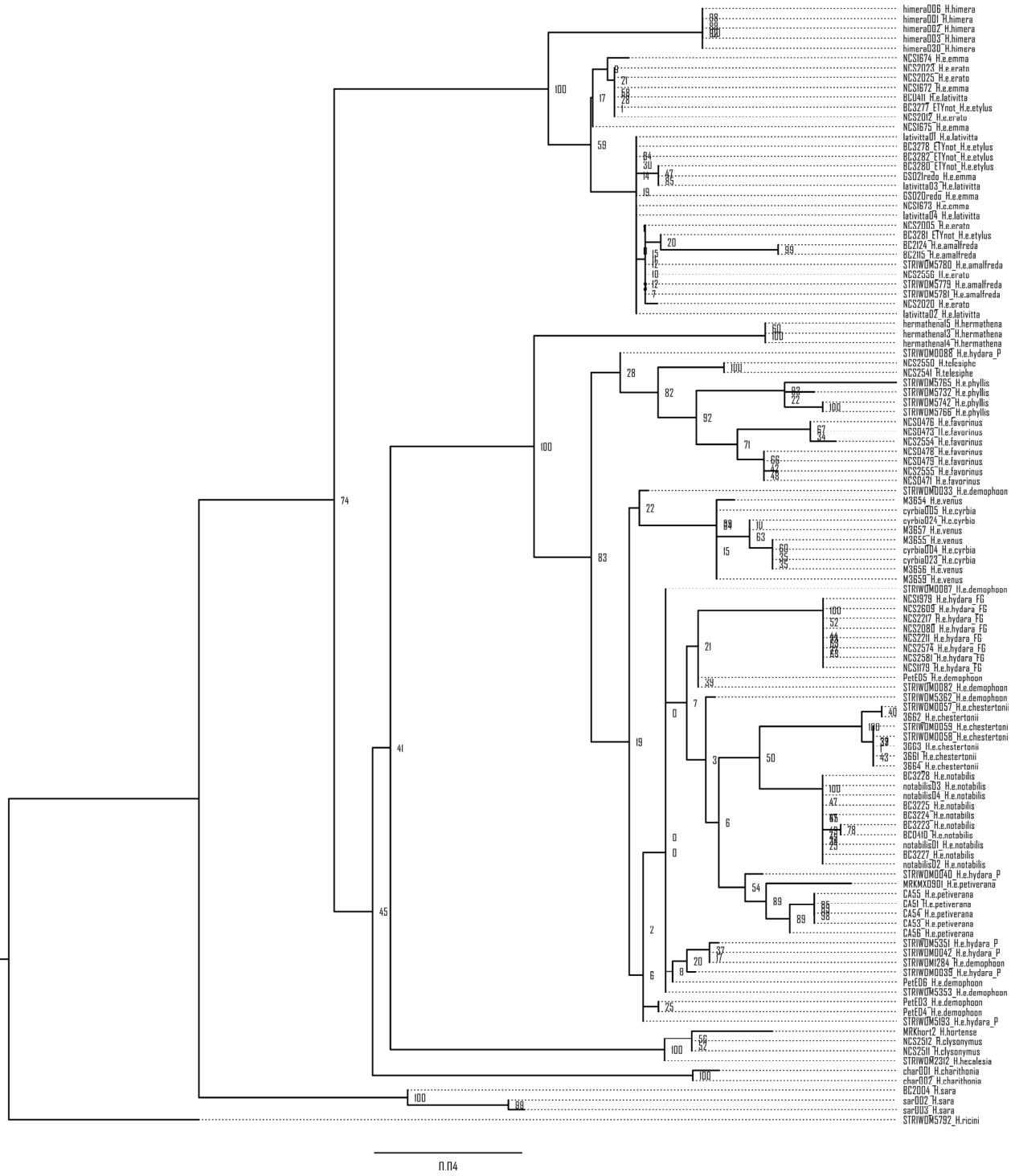


Figure S 26. Maximum likelihood tree for the Y2 element near *optix* on chromosome 18 (Herato1801 position 1420912-1422355) for all *H. erato* samples and closely related outgroup species.

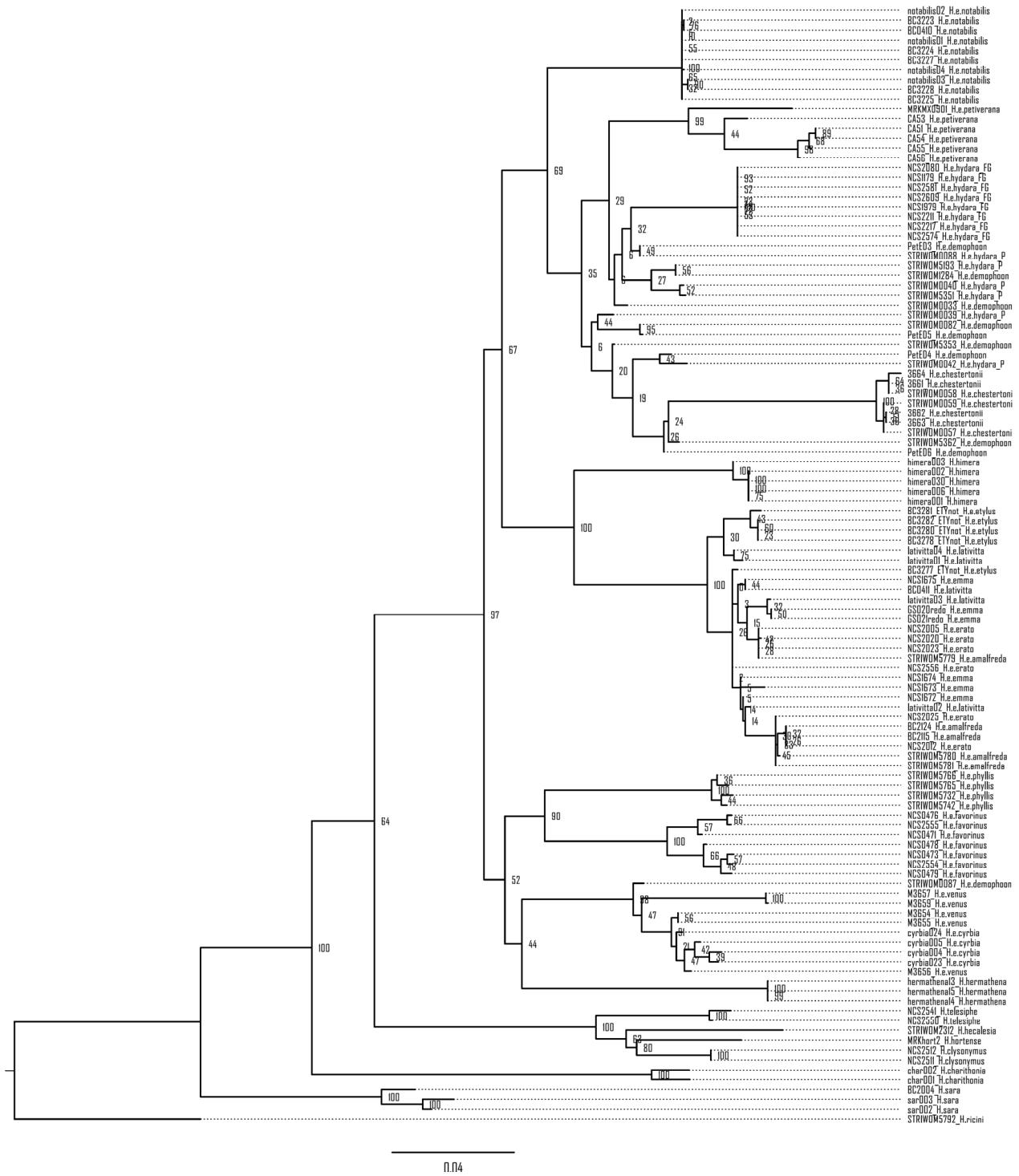


Figure S 27. Maximum likelihood tree for the *D1* element near *optix* on chromosome 18 (Herato1801 position 1412888-1419375) for all *H. erato* samples and closely related outgroup species.



Figure S 28. Maximum likelihood tree for the *D2* element near *optix* on chromosome 18 (Herato1801 position 1422585-1428307) for all *H. erato* samples and closely related outgroup species.

S5.3.3. Comparison of the *optix* locus between *H. erato* and *H. melpomene*

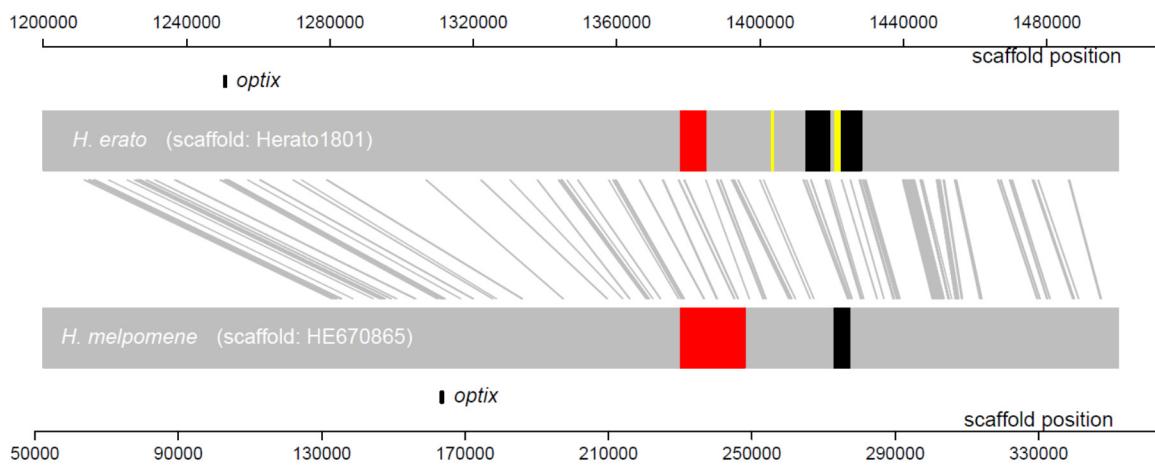


Figure S 29. Comparison of the R (rays; red), Y (band; yellow) and D (dennis; black) regions of *Heliconius erato* to *Heliconius melpomene* near the *optix* gene on chromosome 18. The *H. erato* and *H. melpomene* scaffold including the *optix* gene and the genomic intervals associated with R (Rays) and D (Dennis) were aligned using PROmer selecting for alignments with at least 85 % sequence identity (3). The alignment suggests that *H. erato* and *H. melpomene* have independent (non-overlapping) modules that regulate red color pattern variation.

S5.4. *Cortex*

The *Cr* locus has been found to control variation in yellow/white patterns across several regions of the hindwing¹³. For instance, in *H. e. cyrbia* and *H. e. venus* the hindwing bar is only present on the ventral side, unlike in several other postman *H. erato* races with yellow bars on the ventral and dorsal side. Genomic comparisons of *H. e. venus* and *H. e. cyrbia* to *H. e. hydara*, which lacks any yellow hindwing bar, revealed elevated divergence across large regions across the *Cr* locus and much of the rest of the genome (Figure S 33). This pattern of high divergence limited our ability to resolve an interval at the *Cr* locus associated with a yellow bar that is expressed exclusively on the ventral hindwing. Similarly, *H. e. cyrbia*, which possesses a unique white fringe among hindwing margin not found in any other *H. erato* races, has previously been shown to be controlled by the *Cr* locus. Although comparisons of *H. e. cyrbia* and the neighboring *H. e. venus*, which does not have a white hindwing fringe identified a few narrow regions of divergence across the *Cr* locus, many other narrow peaks of divergence were identified near the *Cr* locus as well as across most of the genome (Figure S 33). Again, this limited our ability to identify specific intervals associated with the presence of the white hindwing fringe. The phylogenetic weighting approach had limited ability to resolve putative regulatory intervals at the *Cr* locus, due to a lack of geographically isolated populations that shared similar *Cr* phenotypes.

S5.4.1. Sliding window population statistics near *cortex*

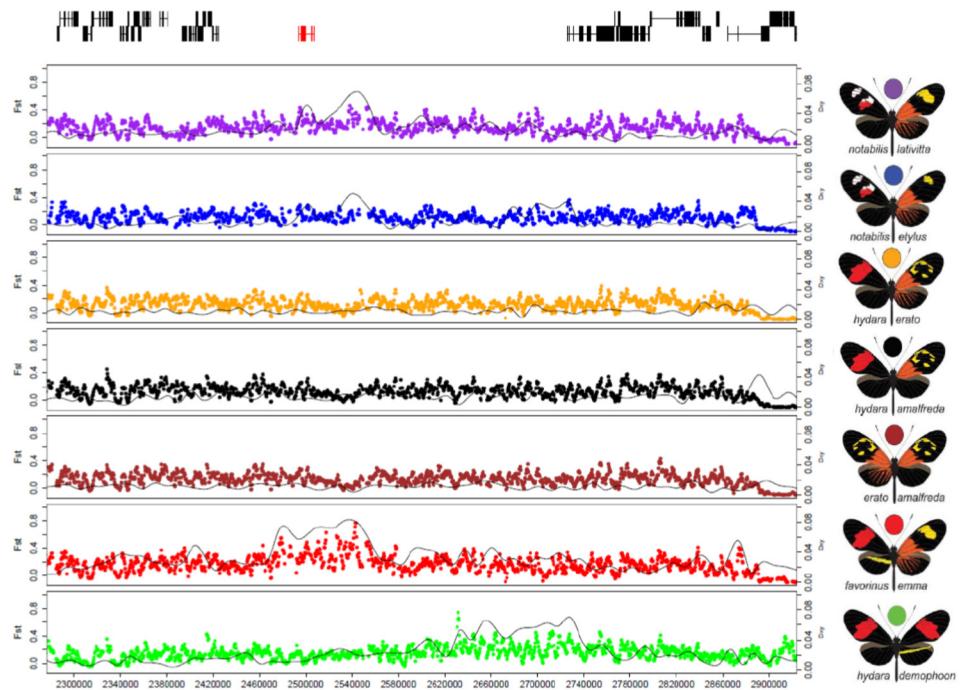


Figure S 30. Sliding window F_{ST} (black line) and D_{XY} (points) near the *cortex* gene on chromosome 15 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

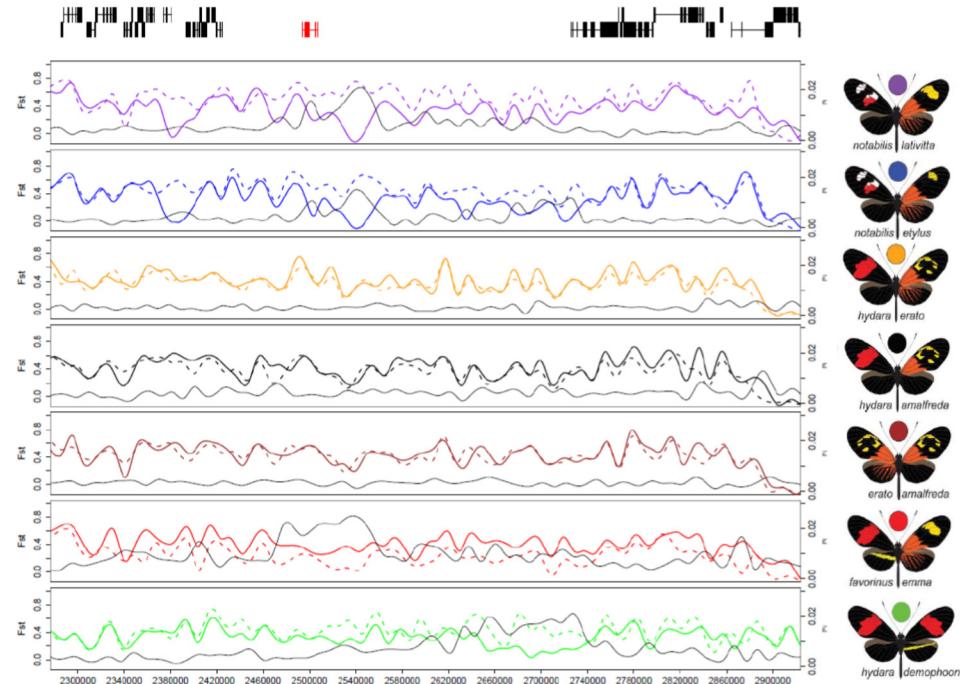


Figure S 31. Sliding window F_{ST} (black line) and nucleotide diversity (π) (colored lines) near the *cortex* gene on chromosome 15 across *Heliconius erato* phenotypic transition zones. Values were calculated for 2 kb windows sliding in increments of 400 bp.

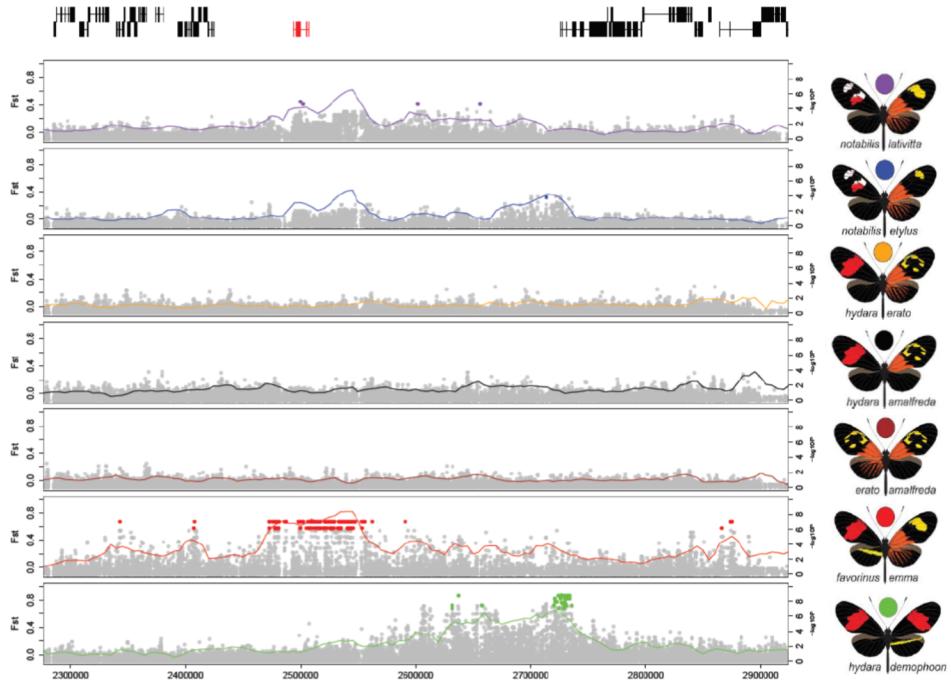


Figure S32. *F_{ST}* (lines; 20 kb window, 5 kb step size) and association (points) analysis near the *cortex* gene on chromosome 15. Colored points represent associations estimated from fixed SNPs.

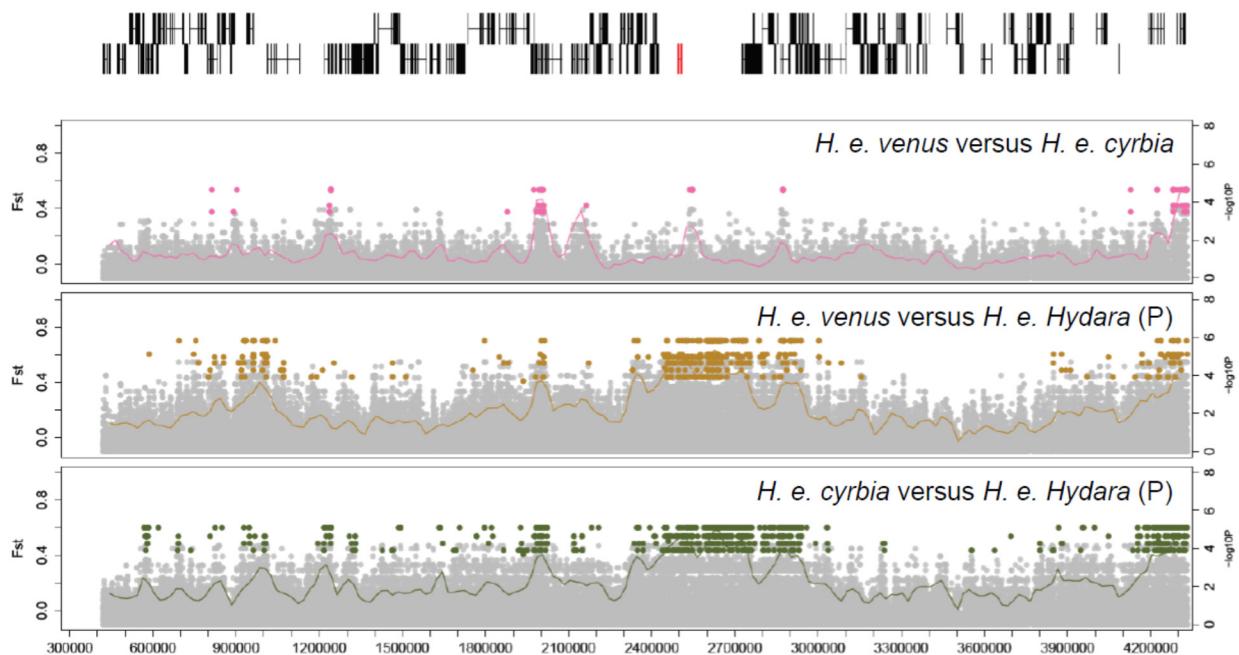


Figure S33. *F_{ST}* (lines; 20 kb window, 5 kb step size) and association (points) analysis near the *cortex* gene on chromosome 15 for comparisons between *H. e. venus*, *H. e. cyrbia* and *H. e. hydara* (Panama). Colored points represent associations estimated from fixed SNPs.

S5.4.2. Maximum likelihood trees for identified color pattern modules near cortex

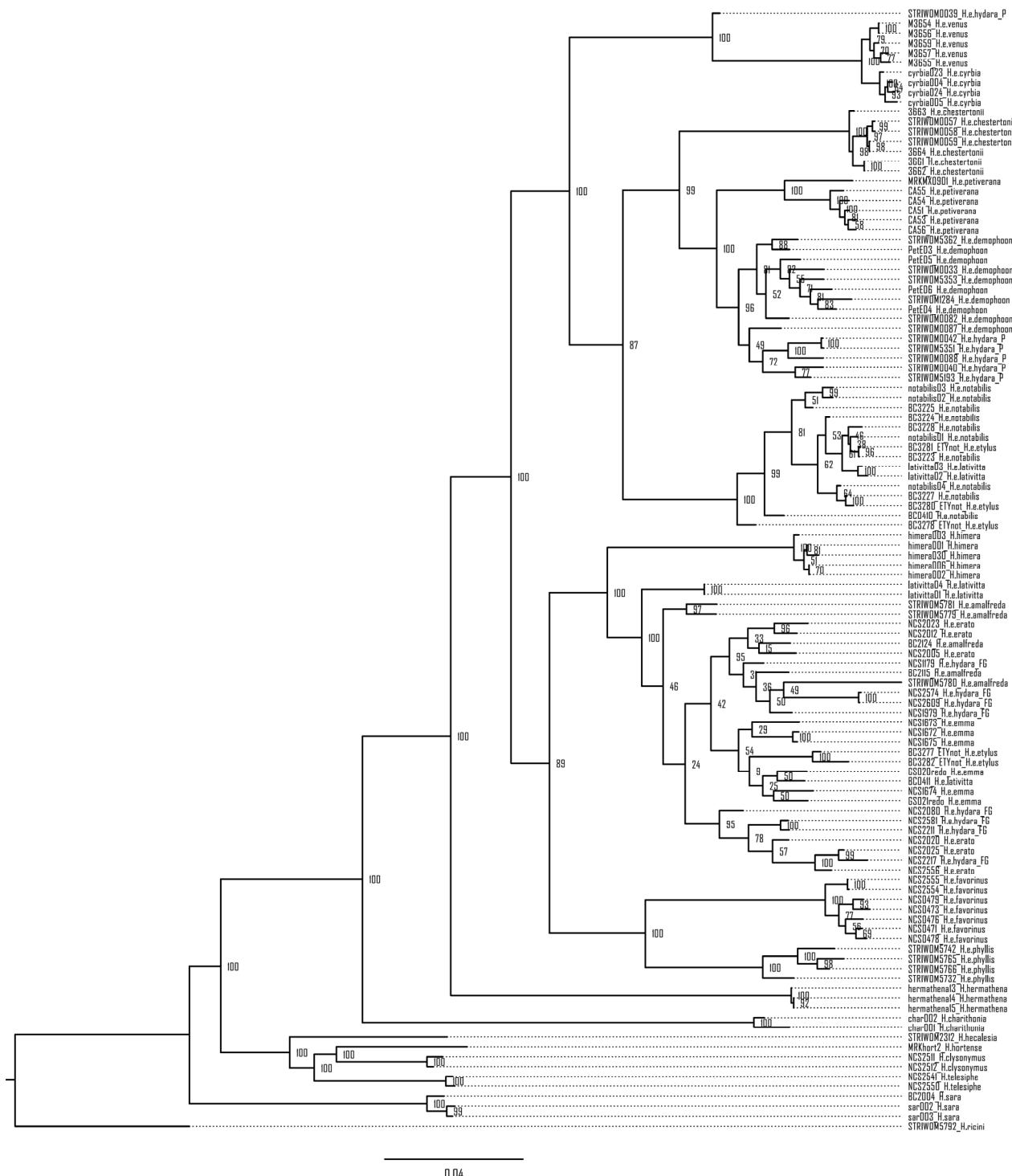


Figure S 34. Maximum likelihood tree for the *Cr1* element near *cortex* on chromosome 15 (Herato1505 position 2053037-2171230) for all *H. erato* samples and closely related outgroup species.

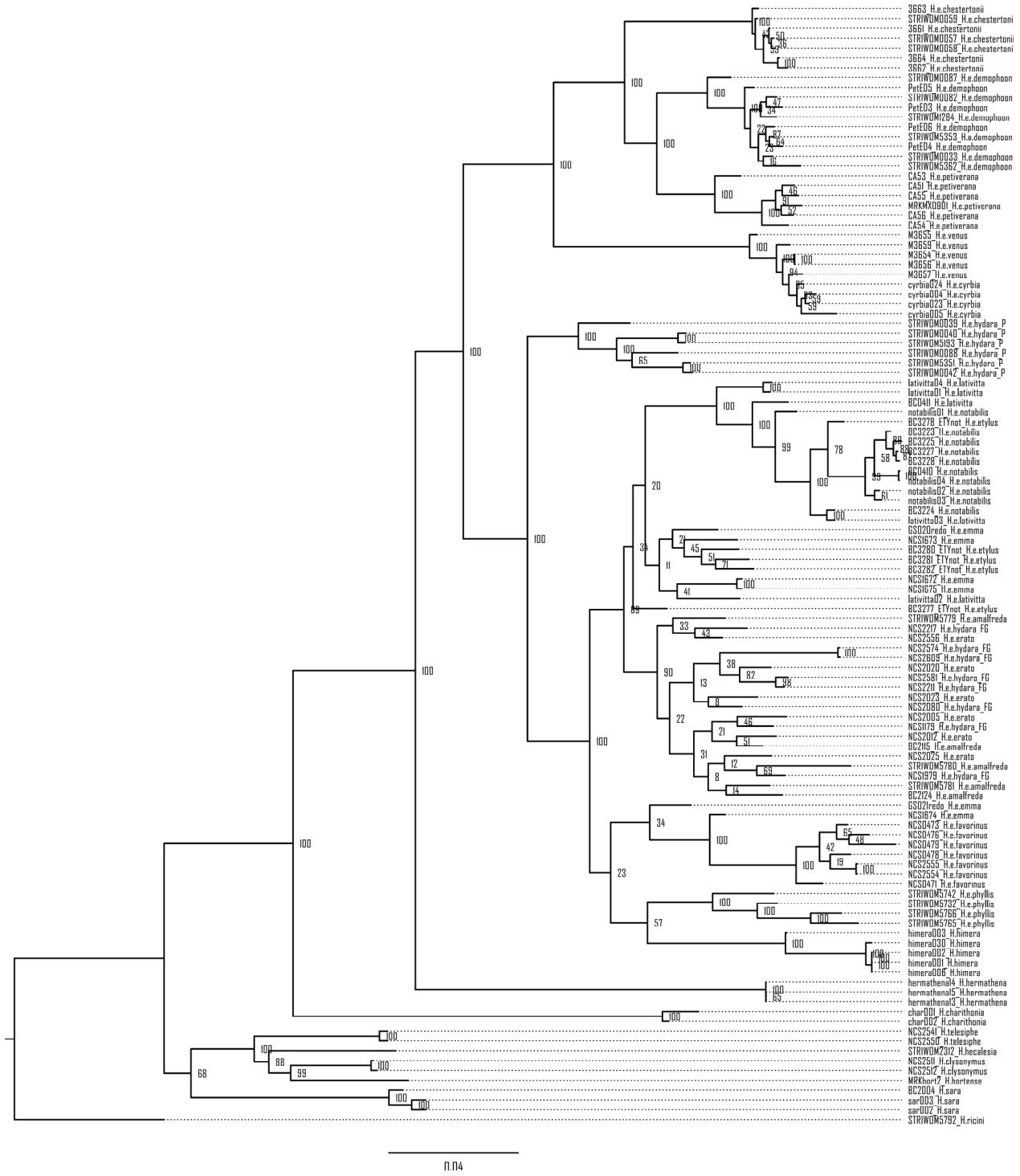


Figure S 35. Maximum likelihood tree for the CrI element near *cortex* on chromosome 15 (Herato1505 position 2211881-2315926) for all *H. erato* samples and closely related outgroup species.

S6. References

1. Stanke, M., Keller, O., Gunduz, I., Hayes, A. & Waack, S. AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res.* **34**, 435–439 (2006).
2. Remmert, M., Biegert, A., Hauser, A. & Johannes, S. HHblits : Lightning-fast iterative protein sequence searching by HMM-HMM alignment. *Nat. Methods* **9**, 173–175 (2012).
3. Haas, B. J. *et al.* De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **8**, 1494–512 (2013).
4. Papanicolaou, A. Just Annotate My genome (JAMg). (2016). at <<https://github.com/genomecuration/JAMg>>
5. Papanicolaou, A. Digital Expression on the Web (DEW). (2016). at <[https://github.com/alpapan/DEW](https://github.com/genomecuration/JAMp)>
6. Heckel, D. G., Gahan, L. J., Liu, Y.-B. & Tabashnik, B. E. Genetic mapping of resistance to *Bacillus thuringiensis* toxins in diamondback moth using biphasic linkage analysis. *Proc. Natl. Acad. Sci.* **96**, 8373–8377 (1999).
7. English, A. C. *et al.* Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. *PLoS One* **7**, e47768 (2012).
8. Simão, F. A., Waterhouse, R. M., Ioannidis, P. & Kriventseva, E. V. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**, 3210–3212 (2015).
9. Rosser, N., Dasmahapatra, K. K. & Mallet, J. Stable *Heliconius* butterfly hybrid zones are correlated with a local rainfall peak at the edge of the Amazon basin. *Evolution* **68**, 3470–3484 (2014).
10. Morgulis, A. *et al.* Database indexing for production MegaBLAST searches. *Bioinformatics* **24**, 1757–1764 (2008).
11. Wallbank, R. W. R. *et al.* Evolutionary novelty in a butterfly wing pattern through enhancer shuffling. *PLoS Biol.* **14**, e1002353 (2016).
12. Frazer, K. A., Pachter, L., Poliakov, A., Rubin, E. M. & Dubchak, I. VISTA: computational tools for comparative genomics. *Nucleic Acids Res.* **32**, 273–279 (2004).
13. Sheppard, P. M., Turner, J. R. G., Brown, K. S., Benson, W. W. & Singer, M. C. Genetics and the Evolution of Muellerian Mimicry in *Heliconius* Butterflies. *Philos. Trans. R. Soc. B Biol. Sci.* **308**, 433–610 (1985).