

Supplementary File 7: CRISPR injection experiments and genotyping primers.

Table S7.1: CRISPR experiments and guides used per species/morph.

Species	sgRNAs	Final concentration ng/μl [sgRNA1(:sgRNAs):Cas9]
<i>H. erato demophoon</i>	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4 , Hera_Cort_gRNA_E6	100:(100):(100):300
	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4	250:(250):500
	Hera_Cort_gRNA_E2	500:500
	Hera_Cort_gRNA_E4	500:500
	Hera_Cort_gRNA_E6	500:500
<i>H. erato cyrbia</i>	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4 , Hera_Cort_gRNA_E6	100:(100):(100):300
	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4	250:(250):500
	Hera_Cort_gRNA_E2	500:500
	Hera_Cort_gRNA_E4	500:500
	Hera_Cort_gRNA_E6	500:500
<i>H. erato hydra</i>	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4	250:(250):500
	Hera_Cort_gRNA_E2	500:500
	Hera_Cort_gRNA_E4	500:500
	Hera_Cort_gRNA_E6	500:500
<i>H. charitonia</i>	Hera_Cort_gRNA_E2 , Hera_Cort_gRNA_E4	500:500
	Hera_Cort_gRNA_E6	500:500
<i>H. melpomene rosina</i>	Hmel_Cort_gRNA_E2, Hmel_Cort_gRNA_E3	250:(250):500
	Hmel_Cort_gRNA_E2	500:500
	Hmel_Cort_gRNA_E3	500:500
<i>H. melpomene plesseni</i>	Hmel_Cort_gRNA_E2, Hmel_Cort_gRNA_E3	250:(250):500
	Hmel_Cort_gRNA_E2	500:500
	Hmel_Cort_gRNA_E3	500:500
<i>H. melpomene cythera</i>	Hmel_Cort_gRNA_E2, Hmel_Cort_gRNA_E3	250:(250):500
<i>H. hecale melicerta</i>	Hmel_Cort_gRNA_E2	250:(250):500
	Hmel_Cort_gRNA_E3	500:500

Time of collection (hours)	Number eggs injected	Number Hatched	Number Puape	Number of Adults	Hatched to Adult Ratio
2-4 hrs	360	135	56	43	11.94444444
1-3 hrs	206	73	45	37	17.96116505
2-4 hrs	134	42	21	16	11.94029851
2-4 hrs	111	46	19	17	15.31531532
2-3 hrs	123	26	17	10	8.130081301
2-4 hrs	236	98	53	43	18.22033898
1-3 hrs	622	180	97	61	9.807073955
1-3 hrs	114	36	18	13	11.40350877
1-4 hrs	221	81	40	24	10.85972851
2-3 hrs	288	71	31	26	9.027777778
1-4 hrs	515	191	86	46	8.932038835
1-3hrs	63	14	8	6	9.523809524
1-3hrs	61	23	13	9	14.75409836
1-4 hrs	68	13	7	6	8.823529412
2-4hrs	120	84	21	15	12.5
2-4hrs	109	61	40	34	31.19266055
1-3hrs	181	56	43	39	21.54696133
1-3hrs	98	41	19	12	12.24489796
1-4hrs	55	30	18	12	21.81818182
1-3hrs	87	56	14	9	10.34482759
1-4hrs	124	93	48	37	29.83870968
1-3hrs	86	37	30	21	24.41860465
1-3hrs	151	64	23	16	10.59602649
1-3hrs	104	51	21	14	13.46153846
1-2hrs	56	32	15	11	19.64285714

Number of adults with phenotype	Wing mutant phenotype ratio from hatched eggs	Wing mutant phenotype ratio from emerged adults
1	0.30%	2.30%
5	2.40%	13.50%
0	0	0
0	0	0
0	0	0
1	0.42%	2.32%
9	1.45%	14.80%
0	0	0
0	0	0
0	0	0
3	0.58%	6.50%
0	0	0
0	0	0
0	0	0
2	1.67%	13.33%
0	0	0
1	0.56%	2.56%
0	0	0
1	1.81%	8.30%
1	1.80%	11.11%
2	1.60%	5.41%
0	0.00%	0.00%
2	1.32%	12.50%
5	4.81%	35.70%
1	1.79%	9.09%

Table S7.2: Sequences for guides yielding succesful phenotypes and associated genotyping primers

Species	sgRNA Name	Target Sequence (5' > 3')	Genotyping Primers (5' > 3')
<i>Heliconius erato</i>	Hera_Cort_gRNA_E2	GGGGCGCTACGAACCTATCTAGG	ATGATATTGAGTTTTGCTAAATCAAGCC GAGTGGCGGAATTCAACGCC
	Hera_Cort_gRNA_E4	GGCTCCTCCAACCTCCATTATGG	CGAGTAAAAGTTCTCCTCTGGG CGTGTTGGTGGCRGCACTTG
	Hera_Cort_gRNA_E6	CCAGCAGTATCGCTTCTCTCACA	-
<i>Heliconius melpomene</i>	Hmel_Cort_gRNA_E2	GCGTTTCGCTGAAAGAATCTCGGG	TGTACATCAAAGGCCGCATG CCTCCGTTTCCCACTTTTCG
	Hmel_Cort_gRNA_E3	GGTCCAGAATGCTGTCTGCTGAGG	TTGCGTAGCTGTATGTAGGCA AAGCCTTCGATCTGGAGTCA

