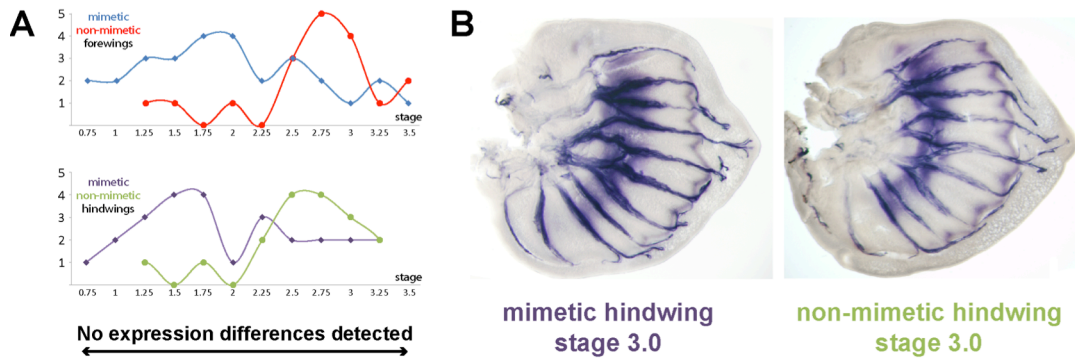
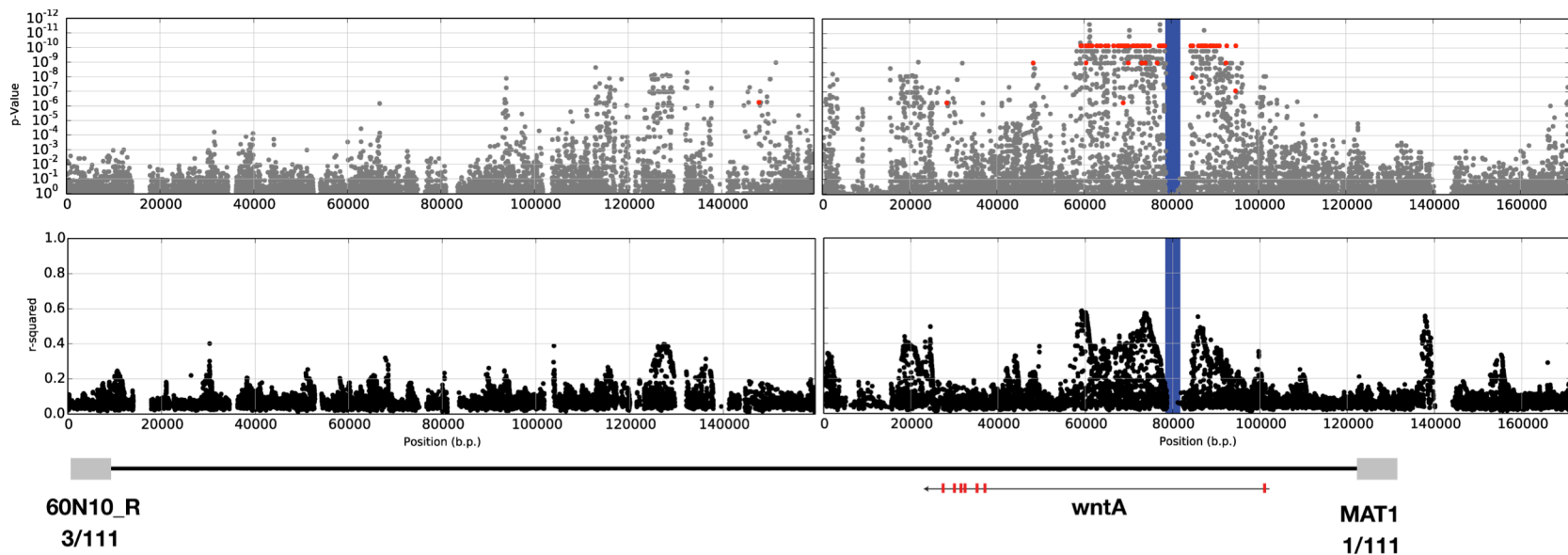


Supplementary Figure 1: Linkage map of *Limenitis* wing patterning chromosome showing position of the zero-recombinant interval between Mat1 and the mapped BAC-end sequence, 6010NR. Co-dominant nuclear markers, which allowing syntenic comparisons to other Lepidopteran systems (e.g. – *Heliconius*, *Bombyx*), are displayed in red.

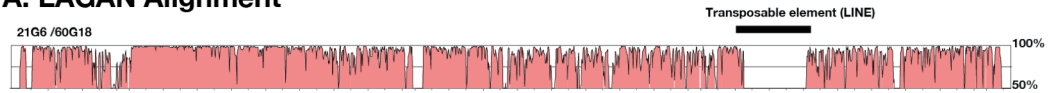


Supplementary Figure 2: Identical *WntA* expression patterns in larval wing disks of banded and unbanded *Limenitis* morphs. **A.** Developmental sampling of the larval wing disks stages¹ used for *WntA* in situ hybridization. Stage-by-stage comparisons revealed no spatial differences in *WntA* expression domains across the fifth instar stage. **B.** Example comparing the expression of *WntA* in hindwings of identical stage of banded (non-mimetic) and unbanded (mimetic) morphs. The tracheal signal is an artifactual, non-specific staining obtained with most riboprobes at certain stages.

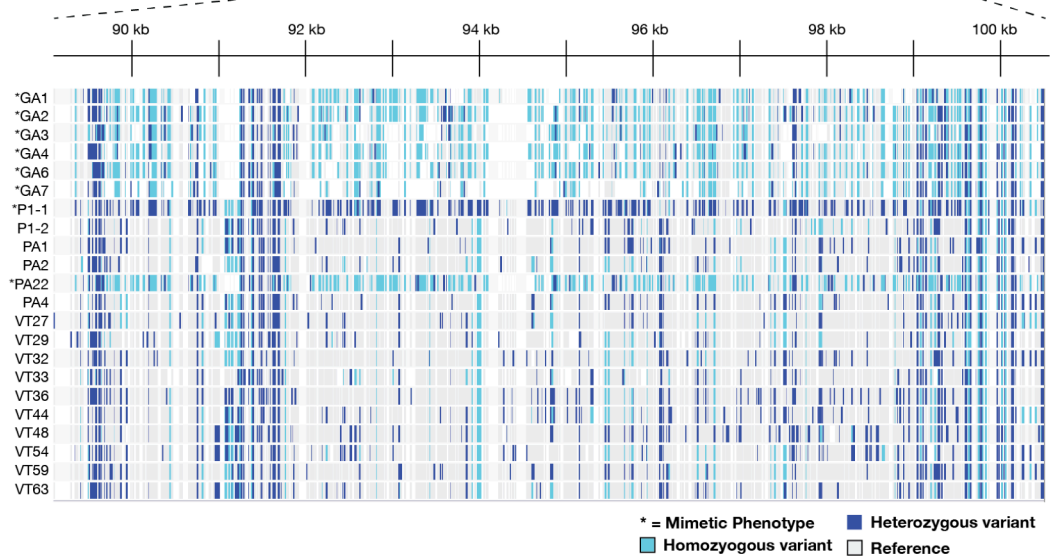


Supplementary Figure 3: SNP associations with mimetic polymorphisms within *Limenitis*. The entire zero-recombinant color patterning interval in *Limenitis* (represented as black line below plots) is indicated. Numbers of recombinants for each marker are indicated. The zero-recombinant interval is represented in two BAC sequences (60N10 left; 21G6 right). Variable nucleotides (grey) mapped by position (x-axis) against the p-value for the Fisher's exact test of allelic association with phenotype (y-axis); SNPs fixed between mimetic and non-mimetic individuals are shown in red. Pairwise linkage disequilibrium, calculated using r^2 (y-axis) between SNPs in 500bp sliding windows across the interval, is plotted for each SNP by position (x-axis) as black dots; the highlighted blue interval corresponds to the position of a Long Interspersed Element (LINE) retrotransposon fixed between mimetic and non-mimetic individuals

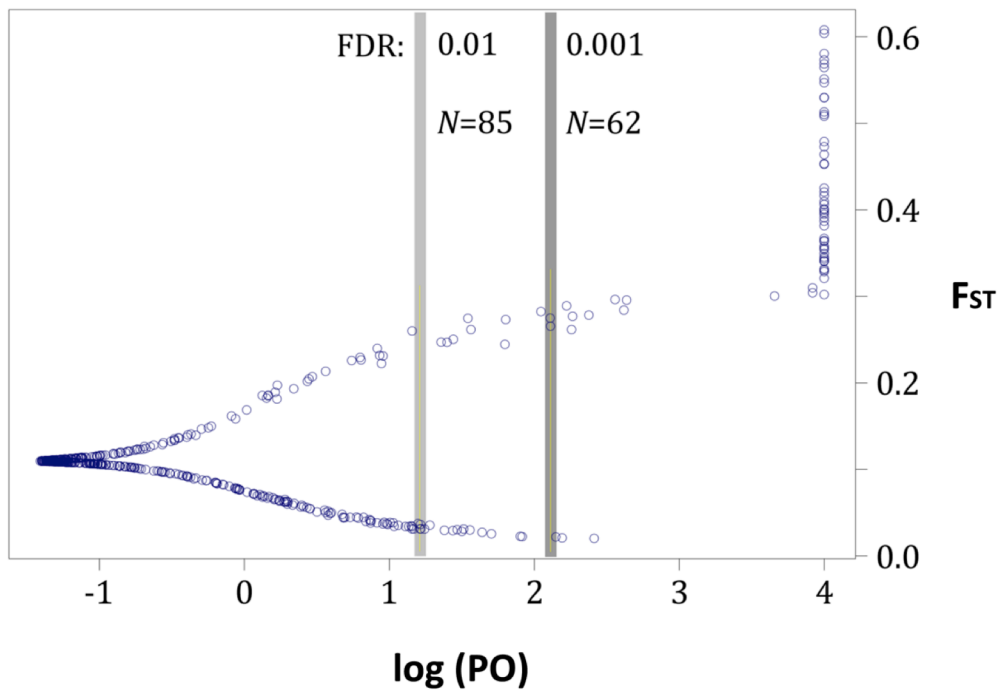
A. LAGAN Alignment



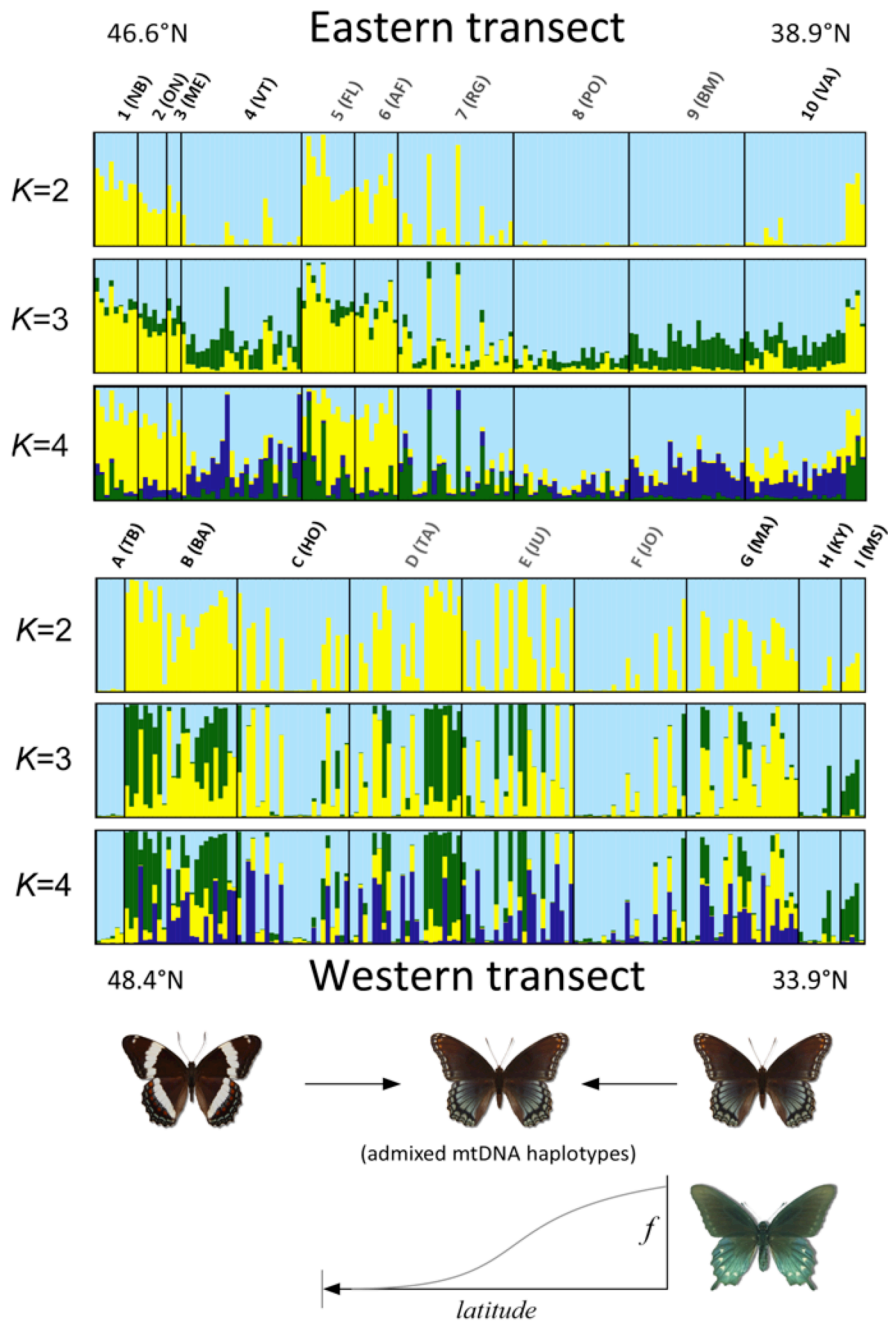
B. BAC 60 G18



Supplementary Figure 4: Genotyping the LINE element. **A.** LAGAN alignments of the two alternative BAC sequences, corresponding to the mimetic (21G6) and non-mimetic (60G18) revealed an approximately 10kb gap of poor alignment which corresponded to a long interspersed transposable element (LINE) present in the 21G6 BAC that was not present in the 60G18 BAC. **B.** Whole genome resequencing and alignment to 60G18 (the LINE-absent BAC) enabled us to genotype the BAC among GA, PA, and VT populations, as well as parents of our mapping brood (P1-1 and P1-2). The numerous homozygous variant nucleotides over this interval in the GA populations indicates homozygosity for the alternative sequence (21G6) containing the LINE, which was also detected in the mimetic individual from PA.



Supplementary Figure 5: Bayesian outlier analysis of the combined transects based on 490 AFLP loci (min. allele freq. >0.10).



Supplementary Figure 6: STRUCTURE clustering results ($K=4$, max. likelihood value) for the eastern and western hybrid zone transects. Population labels in light grey represent phenotypically admixed populations.

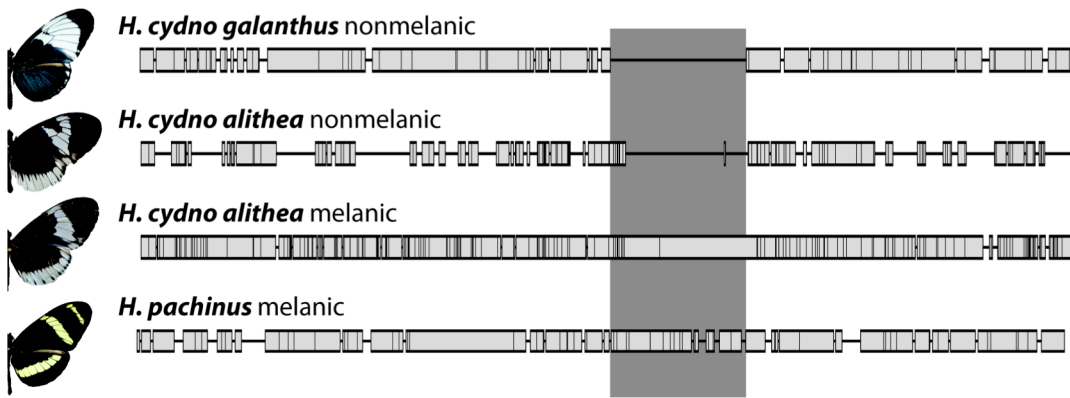
IRES Motif Identification

Mimetic 5'UTR

```
      20          40          60          80
CACCAATTGCCATAACAGCTTCTACCATAAAAAATTATACAGCACAAAACCTTCCAAAAAAGCTTCATTTTGTATAAAAGTTCAATAAAACACAAAAA
100
|          120          140          160          180
AAAATCTTCAACACTTCACAGCACAAAAATTTAAAAAACAATATTTTATTAAACATTTCGTCAAACACAAAAGCACAGAAAAATTTAAAAAATACTA
200
|          220          240          260          280
ATAATTTGAAACTGGCTGGAGAGTAAATAAAAAGGCGGGATTAAAAGAATTCGTTATTTGAAATTGGAAGTGTGTTGGCGCCTGCGACCCGCGACCCGC
300
|
GCGCTGGG
```

Predicted IRES Motif

Supplementary Figure 7: The WntA genomic DNA sequence (~73 kb), was searched using the UTRscan utility furnished by the UTRsite database²³. UTR Scan identified an IRES sequence (accession number U0015) in the mimetic allele in the position shown. The UTRsite lists IRES sequence occurrence to be approximately 0.006 hits/kb.



Supplementary Figure 8: Alignment of resequencing data for *H. cydno galanthus*, *H. pachinus*, and wing pattern morphs of *H. cydno alithea*, mapped using the *Heliconius melpomene* reference assembly showing the presence or absence of structural variation. The position of the causative 1.8Kb deletion in *H. cydno galanthus* and the wing pattern of *H. cydno alithea* with reduced melanin is highlighted in dark grey.

Supplementary Table 1. Abbreviations, gene names, and primer sequences for all codominant loci mapped on the wing patterning chromosome in *Limnitis*.

	Abbv	Gene	Forward	Reverse
1	Vma21	Vacuolar ATPase subunit C	CCT AAT GGC AAA CAA CAG CGA CTT GC	CCT GTT TGT TTC TTC TCT AAA TTC TGT AGG
2	Med20	Mediator complex subunit 20	ATT CTC CAC AAC TCT GAG CAG CCA	GCA TTT GCC ATT ACA CAT GGT CGG
3	Achn1	Acheron, isoform 1	TAT TAC AAG GCG AGG AGA AGC GCA	ATT GCC GCG TAT TTA TTG CGG AGG
4	Wnt7B	Wingless-type MMTV integration site family, member 7B	TGG GTC AAG AGA GAG AGG AAT TGT	GCA CAG AAC ATT CTG CCA ATG TCA CG
5	Gatad1	GATA zinc finger domain-containing protein 1	CCT CCG CAA TAA ATA CGC GGC AAT	GTG GAT TCT TTA CAC CAC GAT CCC
6	Poxn	Paired box neuro protein	GGC CAA GTC TTC TGT CGT AAA T	GTG CTA CAC GTC CAC AAT GTA T
7	ORF2	putative ORF2-encoded protein [Danaus plexippus]	ACC TCA AAT GTG GAG CTT TCT	GAG GTA TGC CAA CAG AGA GAT TAG
8	EH	Juvenile hormone epoxide hydrolase	GTT CTT AAA GCG CAC CCA AAG	CGT TAC AAC TTA AGA GGT CAT ACT AGA
9	Exu	Exuperantia	GTG ACT GAA GCA AAG GTG AAT G	AGA GCA CTA TCC ACT GAC AAA G
10	Mtap_18	S-methyl-5'-thioadenosine phosphorylase	AGG TAC GAT TGC TTA CTT TGA CT	TGT GAT GAG AGG AGG GTG TAT
11	Mtap_13	S-methyl-5'-thioadenosine phosphorylase	GAC GCA CAA GAA GCA TTG AC	GGA ACC AAC CTA TGC ACA ATT TA
12	Ho	Heme oxygenase	AGC GAC ACC CTG GTT AAT G	TCA GAC ATT GTT TCC GCT ATC T
13	Lyzl	Lysozyme-like protein	GCA ACA TTA CTT GCG AAT CAC T	GTG ATA AGG GTC GAA CAC AGA T
14	Reep2	Receptor expression-enhancing protein, isoform 2	ACC GTT TAT TTA TAG GCC GAG AG	TTG GAA GTT AGC GAC TCA ATC A
15	JHEBP	Juvenile hormone esterase binding protein	CTC GAA GGA CTT GTG GAG AAA	CCA GCT GTC TTC TAC TCC TTT AG
16	Mat1	CDK-activating kinase assembly factor MAT1	AAG CCT GTC CGC GTT GTA AGA CTA	CAA ATA ACT GTA CAC GGA AAT TGC TCC
17	WntA	Wingless-type MMTV integration site family, member 1A	CGC CAA CAG ACA CGA ACT GAA CAA	ACA ACA ATG AAG CTG GAC GTA GGG
18	Vldlr	Chitinase synthase 1	TTC GAT TCC TGG TCC ATG GGT TGT	TGG GTA GAC TCT TGG GCA TCT GTT
19	Vldlr	Chitinase synthase 2	AGC AAC TGC TGG ACT GCG ATA TGA	TGG ATT GCC GAC CCA TGG ATA TGT
20	Nup62	Nuclear pore glycoprotein p62	TGT ATG CAT GAG TGA GTG TAT TG	ATA GTG CAG ATA GGA CGC AT
21	BAC End	BAC60n10r	CTC AAG CTT TGT CAT TTA TAA CTT TGC C	GTG AAT GCT TTC CCA AAT TAT TCT CTA CG

Supplementary Table 2. Sampling localities for *Limenitis* and *Heliconius* specimens used for 1) genetic linkage map construction, 2) BAC tile path construction, 3) whole-genome resequencing, and 4) TaqMan® genotyping.

Site	Locality	Lat.	Long.	Species	Mapping	BAC	Genome	TaqMan® Genotyping
<i>Limenitis</i> spp.								
1	Hancock Co., ME, USA	44.5770°	-68.3567°	<i>L. arthemis arthemis</i>	-	-	-	N=24
2	Addison Co., VT, USA	44.0495°	-72.9600°	<i>L. arthemis arthemis</i>	-	-	N=12	N=24
3	Ricketts Glen, Columbia Co., PA, USA	41.3788°	-76.2662°	<i>Intergrades</i>	N=111	N=8	N=6	N=91
4	Shenandoah Co., VA, USA	38.8800°	-78.4303°	<i>L. arthemis astyanax</i>	-	-	-	N=24
5	Putnam Co., GA, USA	33.3066°	-83.4816°	<i>L. arthemis astyanax</i>	-	-	N=12	N=24
<i>Heliconius</i> spp.								
6	Cariblanco, Costa Rica	10° 16' N	84° 11' W	<i>H. cydno galanthus</i>	-	-	1	-
7	Colon, Costa Rica	9° 55' N	84° 15' W	<i>H. pachinus</i>	-	-	1	-
8	Guacimo, Costa Rica	10° 13' N	83° 41' W	<i>H. cydno galanthus</i>	-	-	2	-
9	Guapiles, Costa Rica	10° 13' N	83° 47' W	<i>H. cydno galanthus</i>	-	-	1	-
10	SB La Selva, Costa Rica	10° 26' N	83° 59' W	<i>H. cydno galanthus</i>	-	-	2	-
				<i>H. melpomene rosina</i>	-	-	2	-
11	PN Carara, Costa Rica	9° 47' N	84° 36' W	<i>H. pachinus</i>	-	-	1	-
12	PN Hitoy Cerere, Costa Rica	9° 40' N	83° 2' W	<i>H. cydno galanthus</i>	-	-	1	-
13	PN Manuel Antonio, Costa Rica	9° 24' N	84° 10' W	<i>H. melpomene rosina</i>	-	-	2	-
				<i>H. pachinus</i>	-	-	2	-
14	Puriscal, Costa Rica	9° 51' 0N	84° 19' W	<i>H. melpomene rosina</i>	-	-	2	-
				<i>H. pachinus</i>	-	-	3	-
15	Selva Bananito, Costa Rica	9° 52' N	83° 0' W	<i>H. melpomene rosina</i>	-	-	1	-
				<i>H. pachinus</i>	-	-	2	-
16	Vesta, Costa Rica	9° 43' N	83° 3' W	<i>H. cydno galanthus</i>	-	-	3	-

17	SB Sirena, Costa Rica	8° 28' N	83° 35' W	<i>H. melpomene rosina</i>	-	-	2	-
				<i>H. pachinus</i>	-	-	1	-
18	Pichincha Province, Ecuador	0° 1'S	78° 47' W	<i>H. cydno alithea</i>	-	-	25	-

Supplementary Table 3. Locality data for *Limenitis* specimens genotyped with 12 AFLP primer pairs, and used to assess geographic population structure.

Transect	Site	Code	Locality	Latitude	Longitude	Wing pattern	Subspecies	<i>N</i>
Eastern	1	NB	Holtville, NB, Canada	46.5653°	-66.4619°	Non-mimetic	<i>arthemis</i>	19
	2	ON	Algonquin, ON, Canada	44.7037°	-75.6695°	Non-mimetic	<i>arthemis</i>	16
	3	ME	Hancock Co., ME, USA	44.5770°	-68.3567°	Non-mimetic	<i>arthemis</i>	14
	4	VT	Addison Co., VT, USA	44.0495°	-72.9600°	Non-mimetic	<i>arthemis</i>	24
	5	FL	Finger Lakes National Forest, NY, USA	42.4987°	-76.8133°	intergrades	<i>arthemis-astyanax</i>	11
	6	AF	Allegheny National Forest, PA, USA	42.0483°	-78.8751°	intergrades	<i>arthemis-astyanax</i>	19
	7	RG	Ricketts Glen, Columbia Co., PA, USA	41.3788°	-76.2662°	intergrades	<i>arthemis-astyanax</i>	24
	8	PO	Poconos, Luzerne Co., PA, USA	41.1022°	-75.6864°	intergrades	<i>arthemis-astyanax</i>	24
	9	BM	Blue Mtns, Carbon Co., PA, USA	40.7658°	-75.7338°	intergrades	<i>arthemis-astyanax</i>	24
	10	VA	Shenandoah Co., VA, USA	38.8800°	-78.4303°	Mimetic	<i>astyanax</i>	24
	11	GA	Putnam Co., GA, USA	33.3066°	-83.4816°	Mimetic	<i>astyanax</i>	24
Western	A	TB	Thunder Bay, ON, Canada	48.4068°	-89.2455°	Non-mimetic	<i>arthemis</i>	16
	B	HO	Houghton County, MI, USA	47.0500°	-88.6148°	Non-mimetic	<i>arthemis</i>	24
	C	BA	Bayfield County, WI, USA	46.6651°	-91.1222°	Non-mimetic	<i>arthemis</i>	24
	D	TA	Taylor County, WI, USA	45.2231°	-90.5299°	intergrades	<i>arthemis-astyanax</i>	24
	E	JU	Juneau County, WI, USA	43.8673°	-90.0747°	intergrades	<i>arthemis-astyanax</i>	24
	F	JO	Jo Daviess County, IL, USA	42.3149°	-90.2245°	intergrades	<i>arthemis-astyanax</i>	24
	G	MA	Mason County, IL, USA	40.2271°	-89.9253°	Mimetic	<i>astyanax</i>	24
	H	KY	Jefferson Co., KY, USA	38.1938°	-85.6435°	Mimetic	<i>astyanax</i>	19
	I	MS	Calhoun Co., MS, USA	33.8839°	-89.3227°	Mimetic	<i>astyanax</i>	15

Supplementary Table 4. Selective nucleotide for the 12 AFLP primer pair combinations used to genotype individuals (n=417) across two transects of the phenotypic hybrid zone. F-statistics were calculated using Arlequin (v3.5; Excoffier and Lischer 2010) for 1) all retained loci, 2) all non-outlier loci, and 3) outliers.

<i>EcoRI</i>	<i>MseI</i>	Number of Loci/primer pair	Loci retained for the Bayesian outlier analysis
ACT	CTC	307	59
	CTG	157	31
ACA	CTC	249	36
	CTG	258	28
AAC	CAA	174	35
	CAT	236	47
	CTA	274	50
	CTT	273	48
AT	CTG	115	13
AGG	CAC	267	48
AG	CC	263	61
TT	CC	150	34
Total		2723	490
<i>F_{ST}All</i> (490)	<i>F_{ST}Neutral</i> (428)	<i>F_{ST}Outliers</i> (62)	
0.21	0.09	0.51	