Recent hybrids recapitulate ancient hybrid outcomes

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Genomic outcomes of hybridization depend on selection and recombination in hybrids. Whether these processes have similar effects on hybrid genome composition in contemporary hybrid zones versus ancient, stabilized hybrid lineages is unknown. Here we show that patterns of introgression in a contemporary hybrid zone in *Lycaeides* butterflies predict patterns of ancestry in geographically adjacent, ancient hybrid populations. We find a particularly striking lack of ancestry from one of the hybridizing taxa, *Lycaeides melissa*, on the Z chromosome in both the ancient and contemporary hybrids. The same pattern of reduced *L. melissa* ancestry on the Z chromosome is seen in two other ancient hybrid lineages. More generally, we find that patterns of ancestry in ancient hybrids are remarkably predictable from contemporary hybrids, which suggests selection affects hybrid genomes in a similar way across disparate time scales and during distinct stages of speciation and species breakdown.

Numerous species hybridize [1, 2] or show genomic evidence of ancient admixture [3, 4, 5]. Consequently, many organisms have genomes that are mosaics of chromosomal segments with ancestry from different lineages or species (e.g., [6, 7, 8, 9, 10, 11]). In hybrid zones, where gene flow and hybridization are ongoing, parental chromosomal segments are repeatedly introduced, and hybrids often vary substantially in genome composition [12, 13, 14]. In contrast, when gene flow and hybridization cease, as occurs during hybrid speciation or if one of the hybridizing taxa goes extinct, genome stabilization can occur whereby recombination causes ancestry segment size to decay and ancestry segments fix by drift or selection in the nascent hybrid lineage [7, 15, 16].

In both cases, the genomic mosaic of ancestry segments is shaped by selection, which can include selection against developmental incompatibilities and selection for locally adaptive ancestry combinations [12, 14]. In contemporary hybrid zones with ongoing gene flow, selection results in differential or restricted introgression of some ancestry segments (e.g., barrier loci) [17, 18, 19, 20, 21], whereas selection causes shifts in the frequency of ancestry segments (including fixation) in ancient, stabilized hybrid populations [22, 10, 16]. Changes in genome composition and patterns of linkage disequilibrium during the transition from hybrid zone to stabilized hybrid lineage could alter selection on ancestry segments. Likewise, the primary sources of selection could change from, for example, selection against developmental incompatibilities to selection for novel allele combinations that enhance fitness and persistence in a new environment.

Unfortunately, comparisons of genome composition in contemporary versus ancient hybrids are mostly lacking, especially for natural hybrids (for lab hybrids, see [22, 16]). Consistency between patterns of introgression in contemporary hybrid zones and the genomic mosaic of ancestry in ancient hybrids would suggest a major role for selection in determining hybrid genome composition, and would establish connections between early and late stages of speciation, especially speciation with gene flow and hybrid speciation. For example, consistent patterns would suggest that the same genes or gene regions that prevent the fusion of hybridizing species also experience selection during the origin of

hybrid lineages or species. Such consistency could allow genes or genetic regions of general importance for adaptation and speciation to be identified (e.g., genes with environment-independent effects on fitness) and aid in interpreting patterns of ancestry in cases where ancient admixture occurred but where contemporary hybrids are lacking (e.g., *Homo sapiens*×*H. neanderthalensis* [8]). In contrast, a lack of consistency might imply that outcomes of genome collisions are highly context dependent, and thus difficult to predict (e.g., [23]).

Here, we take advantage of natural hybridization in North American *Lycaeides* butterflies to test the consistency of genome composition between a contemporary hybrid zone and multiple, ancient hybrid lineages that have progressed towards genome stabilization. In North America, *Lycaeides* consists of a complex of four nominal species of small blue butterflies and numerous ancient hybrid populations or lineages [6, 24, 25]. Ancient hybrid populations in the central Rocky mountains and Jackson Hole (hereafter Jackson Hole *Lycaeides*) arose following hybridization between *L. idas* and *L. melissa* within the past 14,000 years [26]. Like *L. idas* (and unlike *L. melissa*), these Jackson Hole *Lycaeides* exhibit obligate diapause and are univoltine; they also use the same larval host plant (*Astragalus miser*) as nearby *L. idas* populations [26, 27, 28]. In contrast, hybrid lineages in the Sierra Nevada and Warner mountains of western North America occupy extreme alpine habitats not used by the parental species and exhibit novel, transgressive phenotypes, such as strong preference for an alpine endemic host plant (*A. whitneyi*) and a lack of egg adhesion to the host substrate [6, 29]. We recently found evidence suggestive of a narrow (1–2 kilometers), contemporary hybrid zone between *L. melissa* and Jackson Hole *Lycaeides* near the town of Dubois, Wyoming at the edge of the Rocky mountains [25]. Butterflies in the putative hybrid zone use feral (i.e., naturalized), roadside alfalfa (*Medicago sativa*) as their host plant (similar to some *L. melissa* populations), and are found in close proximity (along the road) to this nonnative plant.

In the current study, we first verify that the Dubois population constitutes a contemporary hybrid zone between *L. melissa* and Jackson Hole *Lycaeides*; the latter is an ancient hybrid lineage derived from *L. melissa* and *L. idas*. We then use a mixture of partial and whole genome sequence data to compare patterns of introgression in this hybrid zone to the genomic mosaic of ancestry in Jackson Hole *Lycaeides*, and to additional ancient hybrid lineages in the Sierra Nevada and Warner mountains, and thereby quantify the consistency of hybrid genome composition across these cases. We expect less consistency with the Sierra Nevada and Warner mountains lineages as they differ in their ecology and in their taxonomic origin; nonetheless, these lineages represent outcomes of collisions between *Lycaeides* genomes and are useful for understanding the general aspects of what transpires from initial hybridization, through isolation and stabilization. We thus use the contemporary Dubois hybrid zone as a window on the evolutionary process and ask whether similar or very different processes operated during the origin and establishment of multiple, ancient hybrid lineages.

Results

Evidence of a contemporary hybrid zone in Dubois

Patterns of genetic variation across 39,193 SNPs and 23 populations ($N = 835$ butterflies) show that the Dubois, Wyoming population constitutes a contemporary hybrid zone between *L. melissa* and the ancient Jackson Hole hybrid lineage, and thus differs from the ancient Jackson Hole *Lycaeides* populations (Table S1, Figs. 1, 2). Admixture proportions from `entropy` (ver. 1.2; [25]) and a principal component analysis (PCA) of genetic variation show that Jackson Hole *Lycaeides* and the Dubois population are genetically intermediate between *L. idas* and *L. melissa* (Figs. 2B, S1). Whereas Jackson Hole *Lycaeides* form a relatively tight cluster in PCA space (especially within populations) and show similar admixture proportions, butterflies from the Dubois population span the entire genomic gradient from *L. melissa* to Jackson Hole *Lycaeides* (Fig. 2C). This single population, which spans ∼1–2 kilometers (kms) along roadside alfalfa, exhibits greater variation in genome composition than the entirety of Jackson Hole *Lycaeides*, with a range of more than 10,000 km^2 [26]. Also consistent with ongoing hybridization in the Dubois population, these butterflies exhibit elevated linkage disequilibrium (Figs. S2, S3) and intermediate allele frequencies (Figs. S4, S5, S6) at ancestry informative SNPs (i.e., SNPs with an allele frequency difference of 0.3 or more between *L. idas* and *L. melissa*). Taken together, these results demonstrate ongoing hybridization in Dubois between *L. melissa* and nearby Jackson Hole *Lycaeides*, which are themselves a product of ancient hybridization between *L. idas* and *L. melissa* [26].

Patterns of restricted and directional introgression

We fit a Bayesian genomic cline model with `bgc` (ver. 1.04b; [30]) to quantify genome-wide variability in introgression between *L. melissa* and Jackson Hole *Lycaeides* in the Dubois hybrid zone. This method estimates clines in ancestry for individual genetic loci (e.g., SNPs) along a genome-average admixture gradient [31, 14] (Fig. S7). As such, it can be applied in cases in which hybrid zones are confined to a single geographic locality, such as the Dubois population. We focused this analysis on 1164 ancestry informative markers (AIMs; SNPs with allele frequency differences between *L. idas* and *L. melissa* ≥ 0.3). We detected credible variation in patterns of introgression across the genome (defined as cases where Bayesian 95% credible intervals [CIs] for genomic cline parameters did not span zero; Fig. 3). Of the 1164 AIMs, 34 (2.9%) showed credible evidence of restricted introgression (95% CIs for cline parameter $\beta > 0$) and constitute candidates for genomic regions harboring barrier loci between *L. melissa* and Jackson Hole *Lycaeides* ([32]; Figure 3B). 189 of the AIMs (16.2%) had credible evidence of excess Jackson Hole *Lycaeides* introgression relative to genome-average expectations (i.e., directional introgression of Jackson Hole alleles; 95% CIs for cline parameter $\alpha > 0$), and 273 AIMs (23.5%) had credible evidence of excess *L. melissa* introgression (i.e., directional introgression of *L.*

melissa alleles; 95% CIs for cline parameter $\alpha < 0$). Similar results were obtained when only male butterflies were analyzed; males carry two copies of the Z chromosome (Fig. S8).

Genetic loci exhibiting directional or restricted introgression were distributed across the 23 *Lycaeides* chromosomes (Figure 3B). However, randomization tests showed that AIMs with restricted introgression (34 loci with $\beta > 0$) and those with excess Jackson Hole introgression (189 loci with $\alpha > 0$) were found on the Z sex chromosome more than expected by chance ($\beta > 0$, number on Z = 31, x-fold enrichment = 4.70, $P < 0.001$; $\alpha > 0$, number on Z = 67, x-fold enrichment = 1.84, $P < 0.001$) (Fig. 3C,D). This was not true for AIMs with directional introgression of *L. melissa* alleles (273 loci with $\alpha < 0$, number on Z = 37, x-fold enrichment = 0.70, $P = 0.998$). There was little evidence that genetic loci showing exceptional patterns of introgression in Dubois were clustered on genes or other structural genomic features (we did find some evidence of AIMs with credible *L. melissa* introgression being over-represented in coding sequences and proteins; Tables S2, S3 and S4).

Genome composition and genome stabilization in the ancient Jackson Hole hybrid lineage

We next quantified genome-wide variation in the frequency of *L. idas* versus *L. melissa* ancestry segments in each of ten Jackson Hole *Lycaeides* populations (Table S1). This includes populations adjacent to the Dubois hybrid zone (e.g., Bald Mountain [BLD] which is ∼5 km from the Dubois site), and much more distant populations (e.g., Bunsen Peak [BNP], which is on the Yellowstone plateau 173 km from Dubois) (Fig. 2A). We once again focused on the 1164 AIMs. We estimated ancestry segment frequencies using the correlated beta process model implemented in `popanc` (ver. 0.1; [15]). This method is similar to a hidden Markov model and accounts for the expected autocorrelation in ancestry along chromosomes, but allows ancestry frequencies to vary along the genome. The average frequency of *L. idas* ancestry varied from 0.76 (BNP) to 0.51 (BCR), resulting in a northwest-southeast cline in mean genome composition (consistent with [26]) (Figure 4). However, ancestry frequencies varied considerably within and among chromosomes, with 7.7–33.2% of the genome fixed or nearly fixed (frequency ≥ 0.95) for *L. melissa* or *L. idas* ancestry (Figure 4, S9). More of the genome was fixed (or nearly fixed) for *L. idas* ancestry than *L. melissa* ancestry (mean = 10.9% versus 3.5%), and overall rates of fixation were higher on the Z than autosomes (mean = 30.1% on the Z). Similar results were obtained when only male butterflies were analyzed (Fig. S10, S11).

Regions of exceptionally high *L. idas* ancestry frequencies were especially pronounced on the Z chromosome, and this held across all ten Jackson Hole populations (21.3–32.0% of the 225 AIMs on the Z had *L. idas* ancestry frequencies $\geq$0.95). Specifically, randomization tests showed that the 10% of AIMs with the highest *L. idas* ancestry frequencies were found on the Z chromosome 1.28 to 3.32 times more often than expected by chance (Table S5). In contrast, we found little evidence that regions of high *L. melissa* ancestry were over represented

on the Z chromosome (Table S6). Genomic regions with the highest levels of *L. idas* ancestry (i.e., the top 10% of AIMs with the highest *L. idas* ancestry) were in or near (within 1000 bp) genes (observed = 63, x-fold enrichment = 1.20, $P = 0.024$) and in or near gene coding sequences (observed = 55, x-fold enrichment = 1.22, $P = 0.024$) more often than expected by chance (Table S7). A similar pattern held for regions of high *L. melissa* ancestry (Table S8).

Introgression in contemporary hybrids predicts genome composition in ancient Jackson Hole hybrids

Regions of the genome showing exceptional introgression in the contemporary Dubois hybrid zone coincided with genomic regions exhibiting extreme ancestry frequencies in the ancient Jackon Hole hybrid lineage to a much greater extent than expected by chance (Figure 5). For example, AIMs exhibiting restricted introgression in the Dubois hybrid zone (top 10% of AIMs with the highest β values from `bgc`) overlapped with AIMs showing the most extreme ancestry frequencies in Jackson Hole (top 10% closest to 0 or 1) over four times more often than expected by chance (observed = 49, x-fold enrichment = 4.23, $P < 0.001$; Figure 5C). The degree of overlap increased when we considered more extreme cutoffs for the comparison, up to the top 1% of AIMs with the greatest degree of restricted introgression or most extreme ancestry frequencies (x-fold enrichments ranged from 4.23 to 35.42; Figure 5D). Similar patterns were observed when comparing AIMs with evidence of excess directional introgression of Jackson Hole alleles in Dubois (top 10% with highest values or α) and those with the highest (i) *L. idas* (observed = 22, x-fold enrichment = 1.91, $P = 0.0007$) or (ii) *L. melissa* (observed = 26, x-fold enrichment = 2.24, $P < 0.001$) ancestry frequencies in Jackson Hole *Lycaeides* (Figure 5A,D). At least for the case of high *L. idas* ancestry, this strength of this signal of excess overlap was again more pronounced when considering more extreme cutoffs up to the top 1% of AIMs (x-fold enrichments ranged from 1.91 to 16.93; Fig. 5F). In contrast, we found no evidence that AIMs with excess directional introgression of *L. melissa* alleles in Dubois (top 10% with lowest values or α) coincided with those AIMs with the highest *L. idas* or *L. melissa* ancestry frequencies in Jackson Hole *Lycaeides* (Figs. 5B,E,F).

Evidence of consistency (i.e., predictability) of genome composition between contemporary Dubois hybrids and the ancient Jackson Hole hybrids comes from both the autosomes and Z sex chromosome. Specifically, when we repeated the above comparisons with either the 22 autosomes or the Z chromosome alone, we obtained mostly similar results. For example, AIMs showing restricted introgression (top 10% with the highest values for β) and those with the most extreme ancestry frequencies in Jackson Hole *Lycaeides* (i.e., closest to being fixed for *L. idas* or *L. melissa* ancestry) coincided 2.4 times (observed = 23, $P < 0.0001$) and 3.9 times (observed = 37, $P < 0.0001$) more often than expected by chance for the autosomes and Z chromosome, respectively (Fig. 5G,H). Similarly, AIMs showing evidence of excess directional introgression of Jackson Hole alleles in Dubois (top 10%) and the highest frequencies of *L. idas* ancestry in the Jackson

Hole populations coincided 2.2 (autosomes, observed = 21, $P < 0.0001$) and 2.8 (Z, observed = 12, $P = 0.0003$) times more often than expected by chance (Fig. 5G,H). We obtained similar results when defining AIMs as SNPs with an allele frequency difference of greater than 0.2 rather than 0.3 (2126 SNPs) (Figure S12), and when basing our analyses only on male butterflies, which carry two copies of the Z chromosome (Figs. S13, S14).

We next analyzed the potential functional significance of genetic regions harboring the AIMs that were in the top 10% for both restricted introgression in Dubois (high β) and extreme (high *L. idas* or high *L. melissa*) ancestry frequencies in the ancient Jackson Hole *Lycaeides* lineage. We focus on this set of 49 loci as we think these are our best candidates for tagging regions of the genome harboring barrier loci, that is regions of the genome that constitute the basis of (partial) reproductive isolation among these lineages. Twenty-seven of these 49 AIMs were in or near genes (i.e., within 1000 bps of annotated gene boundaries) (in general, these barrier loci were not over-represented in or near specific structural features of the genome; Table S9, also see Tables S10 and S11). These genes exhibited a range of predicted functions (Tables S12, S13), with several genes standing out as being of particular interest. This includes the Z-linked 6-phosphogluconate dehyrdogenase gene, *6-pgd* (Fig. S15), which is associated with cold hardiness and diapause in other insects [33, 34, 35], and constitutes or is linked to a barrier locus in swallowtail butterflies [36, 37, 38, 38]. Four AIMs were in or near two immunoglobulin superfamily genes; these were also on the Z chromosome (Tables S12, S13, Fig. S15). This gene superfamily is crucial for pathogen defense in insects [39], and is associated with reproductive isolation in mice [40]. Also among this set of genes was an autosomal olfactory receptor/odorant binding gene (Fig. S15). Such genes are known to affect host plant use in other butterflies [41, 42]. One of the 49 AIMs was within the autosomal nuclear pore gene *Nup93* (Tables S12, S13). This is part of the nuclear pore complex, which is involved in multiple Dobzhansky-Muller incompatabilities in *Drosophila*. Finally, two AIMs in *armadillo* or *armadillo*-like genes (one autosomal and one Z-linked) that are involved in the Wnt-signalling pathway and affect wing development in *Heliconius* and other butterflies were among this set of candidate barrier loci [43]. A diversity of functions were also predicted for the subset of AIMs with both high directional introgression of Jackson Hole alleles in Dubois and high *L. idas* ancestry in Jackson Hole *Lycaeides* (13 out of the 22 of these were in or near genes); this includes immunoglobulin superfamily genes (Tables S14, S15) (also see Tables S16 and S17).

Extending predictability to additional, ancient hybrid lineages

We next asked whether patterns of introgression in the contemporary, Dubois hybrid zone were also predictive of patterns of ancestry in two additional, ancient hybrid lineages from the Sierra Nevada and Warner mountains of western North America [6, 29, 25]. These ancient hybrid lineages occupy alpine habitat on isolated mountains ∼900–1000 kilometers from Dubois. Past work suggests

that these ecologically distinct populations arose within the past two million years (and perhaps much, much more recently) following hybridization between *L. anna* and *L. melissa* (with a possible contribution from *L. idas* or a close relative of *L. idas*/*L. melissa*) [6, 44, 29, 25]. Using high-coverage, whole genome sequences from *L. anna*, *L. melissa*, *L. idas*, and the Sierra Nevada and Warner mountain lineages, we estimated phylogenetic relationships for the entire genome and for 1000 SNP windows along each chromosome to characterize patterns of ancestry in the ancient hybrids. Based on the results from Dubois and the Jackson Hole populations, we predicted reduced *L. melissa* ancestry on the Z chromosome, and topologies suggesting reduced introgression for the candidate barrier loci (i.e., regions containing the 49 AIMs with restricted introgression in Dubois and extreme ancestry frequencies in Jackson Hole).

Consistent with past work, the whole-genome consensus or "species" tree showed that the Warner mountain population was intermediate between *L. anna* and *L. melissa*/*L. idas*, whereas the Sierra Nevadan population was genetically more similar to *L. anna* (Fig. 6). Nonetheless, trees based on 1000 SNP windows varied across the genome. The species tree was the most common topology overall (29.9%), but several "introgression" trees were also common, especially on the autosomes (Figs. 6, S16, S17). Whereas the trees that differ from the species tree could reflect incomplete lineage sorting, tree topologies were autocorrelated along the genome which suggests that introgression has contributed to these patterns as well (Fig. S17).

The species (i.e., consensus) tree was seen about 30% of the time for autosomal windows, Z chromosome windows and the 1000 SNP windows that contained the 49 candidate barrier loci (Fig. S16). The second most common autosomal tree suggested introgression from *L. melissa* into the Warner mountain lineage (24.6% of topologies). As predicted, this tree was significantly and substantially underrepresented on the Z chromosome (2.3%, x-fold = 0.10, $P < 0.001$) and for the candidate barrier loci (6.1%, x-fold = 0.43, $P = 0.047$) (Fig. 6). In contrast, a tree uniting the two ancient hybrid lineages was rare on the autosomes (4.3%), but commonly observed on the Z chromosome (26.2%, x-fold = 5.36, $P < 0.001$) and for the barrier loci (28.6%, x-fold = 3.62, $P = 0.008$) (Fig. 6). This suggests an ancient shared ancestry between these hybrid lineages that has been especially resistant to gene flow, or alternatively, Z-biased introgression between them. Thus, we find evidence of exceptional patterns of introgression and ancestry in these ancient hybrids consistent with patterns observed in the Dubois hybrid zone and ancient Jackson Hole hybrid lineage. Because the majority of the candidate barrier loci were Z-linked, the signal for these loci is not independent of the signal for the Z chromosome.

Discussion

Biologists have endeavored to connect microevolutionary processes to macroevolutionary changes that have occurred over longer periods of time (e.g., [45, 46, 47]). Here we bridge this gap by showing that microevolutionary patterns and

processes in a contemporary hybrid zone predict the genome composition of ancient, (partially) stabilized hybrid taxa (a macroevolutionary-scale outcome). This mirrors past work in *Helianthus* showing the genome composition of ancient hybrid sunflower species could be predicted from evolution in synthetic hybrids and QTL mapping experiments [48, 22]. However, our results are novel in that they demonstrate that the outcomes of genome collisions can be predicted from natural hybrids as well, and despite differences in the ecological and genomic context of the different hybridization events. Additional work in other organisms where ancient and contemporary hybrids are found is needed to determine the generality of this result (e.g., *Populus* and *Helianthus*; [49, 50, 51]). Beyond this, genomic analyses of multiple lineages at various points along the continuum from hybrid zone to stabilized hybrid species could prove particularly interesting. Such analyses could help distinguish between consistency arising from rapid fixation of ancestry segments versus sustained, consistent selection pressures over long periods of time. More generally, our results suggest that genomic analyses of both hybrid zones and hybrid lineages or species might offer a particularly tractable framework for assessing the ways in which and degree to which speciation (especially hybrid speciation) might be predictable from microevolutionary processes of selection and recombination.

Patterns of parallel or repeatable evolution strongly suggest a major role for the deterministic process of natural selection [52, 53]. Thus, genes and gene regions with restricted introgression in Dubois and extreme ancestry frequencies or phylogenetic relationships in Jackson Hole and the other ancient hybrid lineages represent strong candidates for harboring barrier loci (i.e., speciation genes). Such predictability (i.e., consistency) was highest when considering the Z chromosome, where we detected a lack of *L. melissa* ancestry across the hybrid zone and hybrid lineages. This could be explained by a higher density of selected loci on the Z in general [54], and specifically by an excess of intrinsic incompatibilities arising from epistatic interactions, especially as the hybrid populations generally harbored less *L. melissa* ancestry (lower *L. melissa* ancestry could reflect selection or demographic aspects of the hybridization process). Nonetheless, our ability to predict the genome composition of ancient hybrids, especially the Jackson Hole lineage, held even when excluding the Z. Thus, while our results are consistent with theory and other empirical studies that suggest a disproportionate role for sex chromosomes in speciation (e.g., [55, 54, 21, 14]), this was not the sole factor that made genome composition predictable in this system. Indeed, our analyses highlighted candidate barrier loci on autosomes (e.g., an autosomal olfactory receptor/odorant binding gene and *Nup93*), as well as on the Z chromosome.

In conclusion, the fact that putative barrier loci in the Dubois hybrid zone exhibit exceptional ancestry patterns in ancient hybrid lineages is consistent with the hypothesis that the same genes or gene regions that prevent species fusion during hybridization experience selection during the evolution of hybrid lineages or species. This hypothesis is also supported by studies in other systems showing that incompatibility loci contribute to hybrid speciation [48, 16], and is generally consistent with accumulating data suggesting the same genes

are repeatedly involved in adaptation (e.g., [56, 57]). Thus, seemingly distinct facets of speciation, such as the maintenance of taxonomic boundaries across hybrid zones and the origin of novel biodiversity through admixture, may have a predictable, common genetic basis.

Methods

Genome assembly and annotation

We generated a new, chromosome-scale reference genome for *L. melissa* from information on proximity ligation of DNA in chromatin and reconstituted chromatin. Our previous genome assembly comprised 14,029 scaffolds (total assembly length = 360 mega base pairs [mbps]; scaffold N50 = 65 kilo base pairs [kbps]), which had been joined in a linkage map with 23 linkage groups (*Lycaeides* has 22 autosomes and a ZW sex chromosome system) [58, 59]. In the current study, we improved upon this assembly using DNA sequence data from Chicago and Hi-C libraries [60, 61]. Creation and sequencing of the Chicago and Hi-C libraries was outsourced to Dovetail Genomics. The new sequence data and our old assembly were combined using the HiRise assembler (also outsourced to Dovetail Genomics). The new *L. melissa* genome assembly has a final N50 of 15.5 mbps, and 90% of the genome comprises only 21 scaffolds. A whole genome comparative alignment with `mummer` (version 3.2 with the maximal unique matches setting; [62]) of this new genome assembly with the previously published genome assembly and linkage map shows that each of our previously defined linkage groups corresponds with one (or in one case two) of the new, large scaffolds.

We annotated structural and functional features of the new *L. melissa* genome using the `maker` pipeline (version 2.31.10) [63, 64]. This pipeline uses repeat masking, protein and RNA alignment and *ab initio* gene prediction to perform evidence-based gene prediction, which generates annotations that are supported by quality scores. Prior to using `maker`, we identified repeats in the *L. melissa* genome using `repeatscout` (version 1.0.5) [65]. This program identifies repeat elements, including tandem repeats and low complexity elements, and removes them from the genome. We took this approach to avoid missing repeat elements not already in standard data bases. We provided this *de novo* repeat library to `maker`, and used this along with `repbase` in `repeatmasker` to mask repetitive elements in the genome. `maker` can use protein and RNA sequence data for genome annotation. Since we lacked protein sequences for *L. melissa*, we downloaded 28 protein sequence fasta files from 15 butterfly species (see Table S18) from `LepBase` (version 4) [66]. We concatenated these fasta files to generate a protein sequence data base for `maker`. We used data from 24 *L. melissa* transcriptomes (Forister *et al.*, manuscript in prep.) as additional evidence for genome annotation. We first used `trimgalore` (version 2.6.6, `https://github.com/FelixKrueger/TrimGalore`) for adapter trimming and quality filtering of paired-end RNA sequences. We then used these trimmed

reads to generate a *de novo* transcriptome assembly with `trinity` (version 2.6.6) [67, 68]. The assembled transcriptome was passed to `maker`.

We ran two rounds of the `maker` pipeline. We first ran `maker` without using any information from *ab initio* gene predictors (e.g., `augustus`), to generate *de novo* gene models for the *L. melissa* genome. We then ran the `maker` pipeline again and used the gene models from the first run to train two gene predictors: `augustus` and `snap`. We ran `snap` (version 2006-07-08) [69] by using models with AED scores of 0.25 or better and a length of 50 or more amino acids. We ran `augustus` (version 3.3) with the insect predictions [70]. We then used both of these sets of gene predictions in the second run of `maker`. We then used the output from `maker` to obtain functional annotations of the *L. melissa* genome. We assigned putative gene functions by using `blastp` to query the `maker` output against the UNIPROT/SWISSPROT database [71]. We also used `interproscan` [72] to add protein and gene ontology information to gene model. The final annotation included 11,247 putative genes, 48,765 putative coding sequences, and 8893 UTR sequences.

DNA sequencing, alignment, and genetic variant detection

We analyzed partial genome sequences from 835 *Lycaeides* butterflies from 23 populations: eight *L. melissa* populations (N = 238 butterflies), four *L. idas* populations (N = 156 butterflies), 10 Jackson Hole *Lycaeides* populations (N = 326 butterflies) and the Dubois hybrid zone (N = 115 butterflies) (Table S1). The sequence data from 643 of these butterflies was previously described in a study of admixture across the *Lycaeides* species complex [25]. Data from 192 of the butterflies were generated for the current study, and this includes many (but not all) of the Dubois individuals. We extracted DNA, generated genotyping-by-sequencing (GBS) libraries and sequenced these libraries following the protocols described in [73]. The GBS libraries were sequenced on an Illumina HiSeq 2500 (100 bp, single-end reads) by the Genome Sequencing and Analysis Facility at the University of Texas (Austin, TX).

We used `bwa` (version 0.7.17) to align the GBS sequences from 835 individuals to the draft *L. melissa* genome by using the `mem` algorithm [74, 75]. We ran `bwa mem` with a minimum seed length of 15, internal seeds of longer than 20 bp, and only output alignments with a quality score of $\geq$30. We then used `samtools` (version 1.5) to compress, sort and index the alignments [76]. We used `samtools` (version 1.5) and `bcftools` (version 1.6) for variant calling. For variant calling, we used the recommended mapping quality adjustment for Illumina data (`-C 50`), skipped alignments with mapping quality less than 20, skipped bases with base quality less than 15, and ignored insertion-deletion polymorphisms. We set the prior on SNPs to 0.001 (`-P`) and called SNPs when the posterior probability that the nucleotide was invariant was $\leq$ 0.01 (`-p`). We filtered the initial set of variants to retain only those SNPs with sequence data for at least 80% of the individuals, a mean sequence depth of 2 per individual, at least 4 reads of the alternative allele, a minimum quality score of 30, a minimum (overall) minor allele frequency of at least 0.005, and no more than 1% of the reads in the reverse

orientation (this is an expectation for our GBS method). We further removed SNPs with excessive coverage (3 standard deviations above the mean) or that were tighly clustered (within 3 bp of each other), as these could reflect poor alignments (e.g., reads from multiple paralogs mapping to the same region of the genome). Finally, because we combined data from two different sequencing runs, we also removed any SNPs with a difference in sequence coverage between the published and new data that was more than half the mean coverage for the two data sets combined. This left us with 39,139 SNPs for downstream analyses.

Estimating genotypes, admixture proportions, population allele frequencies, and linkage disequilibrium

We used the admixture model from `entropy` (version 1.2) [25] to obtain Bayesian estimates of genotypes and admixture proportions. This analysis was based on the full data set of 835 individuals and 39,139 SNPs. The admixture model in `entropy` is similar to that in `structure` [77], but differs by accounting for uncertainty in genotypes arising from limited sequence coverage and sequence errors, and by allowing simultaneous estimation of genotypes and admixture proportions [25]. We fit the model with $k \in \{2 \ldots 5\}$ source populations. For each value of k, we ran three Markov chain Monte Carlo (MCMC) chains, each with 15,000 MCMC iterations, a burnin of 5000 iterations and a thinning interval of 5. We used assignments from a discriminant analysis of principal components to initialize the MCMC algorithm; this speeds convergence to the posterior and avoids label switching during MCMC without affecting the posterior probability distribution [25]. We obtained genotype estimates as the posterior mean allele count for each individual and locus across chains and values of k (i.e., this integrates over uncertainty in the number of hypothetical source populations). We focused on admixture proportions for $k = 2$, as we were interested in the two nominal species and hybrids between them. We summarized patterns of population structure and admixture across the sampled populations and individuals based on these admixture proportions and a principal component analysis (PCA) of the genotypic data. We performed the PCA in `R` on the centered but not standardized genotype matrix with the `prcomp` function.

We used the expectation-maximization (EM) algorithm implemented in `estpEM` (version 0.1) [53] to estimate allele frequencies for each SNP (39,139 SNPs) and population (N=23 populations). The EM algorithm estimates allele frequencies while allowing for uncertainty in genotypes [78, 53]. We used the genotype likelihoods calculated with `bcftools` for this analysis, and ran the algorithm with a convergence tolerance of 0.001 and allowing for 20 EM iterations. We used these allele frequency estimates to designate ancestry informative SNPs/markers (AIMs) as those with an allele frequency difference $\geq$0.3 between *L. melissa* (mean of BST, SIN, CDY, and CKV) and *L. idas* (mean of KHL, SDC, and SYC) (population IDs are defined in Table S1).

Finally, we calculated a metric of linkage disequilibrium (LD), the Pearson correlation coefficient between genotypes at pairs of loci, for all pairwise combinations of AIMs in each population where 20 or more butterflies were collected.

Our goal was to ask whether LD was elevated in the Dubois hybrid zone, as predicted by theory (e.g., [79]). We first polarized genotypes such that positive LD (i.e., positive Pearson correlations) coincided with an association between coupling alleles, that is alleles more common in *L. idas* or *L. melissa*, whereas negative LD (i.e., negative Pearson correlations) coincided with associations between repulsion alleles, that is positive associations between *L. melissa* and *L. idas* alleles. Ongoing hybridization should cause a shift towards higher positive estimates of LD, even for unlinked markers (via admixture LD). Correlations were calculated in `R` (version 3.5.1).

Estimating cline parameters in the Dubois hybrid zone

We fit Bayesian genomic clines for each of the 1164 AIMs using `bgc` (ver. 1.04b; [30]) to quantify genome-wide variability in introgression between *L. melissa* and Jackson Hole *Lycaeides* in the Dubois hybrid zone. This method estimates clines in ancestry for individual genetic loci (e.g., SNPs) along a genome-average admixture gradient [31, 14]. As such, it can be applied in cases where hybrid zones are confined to a single geographic locality, such as the Dubois population. Deviations between genome-average introgression and introgression for each locus are measured with two cline parameters, α and β. Cline parameter α denotes an increase (for positive values of α) or decrease (for negative values of α) in the probability of ancestry from reference (parental) species 1 relative to null expectations from an individual's hybrid index. Genomic cline parameter β describes an increase (positive values) or decrease (negative values) in the rate of transition from parental species 0 ancestry to parental species 1 ancestry along the genome-wide admixture gradient. When placed in a geographic context, α is equivalent to twice the shift in cline center, and β measures the decrease (or increase when $\beta < 0$) in cline width relative to the average [79, 14]. Cline parameters can be affected by genetic drift and selection in hybrids [32, 14]. However, in the absence of major geographic barriers to gene flow, high positive values of β (i.e., the equivalent of narrow clines in a geographic context) are most readily explained by selection.

We fit the `bgc` genomic clines model for the 115 *Lycaeides* butterflies from the Dubois hybrid zone. We used *L. melissa* (LAN, SIN, and CKV; N = 131 butterflies) and Jackson Hole *Lycaeides* (BLD and FRC; N = 94 butterflies) as reference or parental species for the analysis. These specific populations were chosen as the source populations because they were nearest to the Dubois hybrid zone. Genotype likelihoods from `bcftools` were used as input for the analysis (the model fit incorporates uncertainty in genotype as captured by the genotype likelihoods). We ran the analysis using only the 1164 AIMs. We fit the model using MCMC, with five chains each with 25,000 iterations, a 5000 iteration burn-in and a thinning interval of 5. We inspected the MCMC output to assess convergence of chains to the stationary distribution and combined the output of the five chains. We repeated this analysis with only males and with AIMs defined as those SNPs with an allele frequency difference of $\geq$0.2 between *L. melissa* and *L. idas* (2126 SNPs) to assess the robustness of our results.

We defined credible deviations from null expectations given genome-wide admixture as cases where the 95% credible intervals (specifically the equal-tail probability intervals) for α or β for a given locus (AIM) excluded 0. We focused specifically on cases of credible directional ($\alpha \neq 0$) or restricted ($\beta > 0$) introgression relative to the genome-wide average. We used randomization tests to ask whether (and to what extent) such loci were over (or under) represented on the Z chromosome or in or near (within 1000 bp of) certain annotated structural features of the genome, such as genes, coding sequences, transposable elements and annotated protein sequences or motifs. Null expectations were derived from 1000 permutations of cline parameter estimates across the 1164 AIMs.

Analysis of population ancestry in Jackson Hole-*Lycaeides*

We estimated ancestry segment frequencies across the genome for each of the ten Jackson Hole ancient hybrid populations using the correlated beta process model implemented in `popanc` (ver. 0.1; [15]). This method is similar to a hidden Markov model and accounts for the expected autocorrelation in ancestry along chromosomes, but allows ancestry frequencies to vary along the genome. It is particularly well suited for cases where genome stabilization has begun but is not yet complete [15]. We ran `popanc` for each of the Jackson Hole populations to estimate the frequency of *L. idas*-derived alleles along the genome. We used a window size of 3 SNPs and focused on the 1164 AIMs. We set SYC and KHL as representative of the *L. idas* parental species, and BST, SIN, CDY and CKV as the putative *L. melissa* parents. Maximum likelihood allele frequency estimates from estpEM were used as input for the program/analysis. We ran the MCMC analysis for each population with a 10,000 iteration chain, a 5000 iteration burn-in and thinning interval of 10. We based on inferences on point estimates (posterior mean) of *L. idas* ancestry frequencies for individuals populations or on averages of these estimates across all ten populations. We repeated this analysis with only males and with AIMs defined as those SNPs with an allele frequency difference of $\geq$0.2 between *L. melissa* and *L. idas* to assess the robustness of our results. Randomization tests were used to ask whether and to what extent AIMs with the highest *L. idas* or *L. melissa* ancestry frequencies (top 10% in each case) were over-represented on the Z chromosome or in or near certain structural features of the genome as described above for the Dubois hybrid zone analyses.

Quantifying consistency in genomic outcomes of hybridization between contemporary and ancient hybrids

We next tested for excess overlap between AIMs showing the most extreme ancestry frequencies in the ancient Jackson Hole hybrids and those with the greatest deviations from genome-wide average introgression in the contemporary Dubois hybrid zone. We focused on the following five comparisons: (i) excess introgression of Jackson Hole alleles in Dubois ($\alpha > 0$) and high *L. idas* ancestry in Jackson Hole, (ii) excess introgression of Jackson Hole alleles in Dubois ($\alpha >$

0) and high *L. melissa* ancestry in Jackson Hole, (iii) excess introgression of *L. melissa* alleles in Dubois ($\alpha < 0$) and high *L. idas* ancestry in Jackson Hole, (iv) excess introgression of *L. melissa* alleles in Dubois ($\alpha < 0$) and high *L. melissa* ancestry in Jackson Hole, and (v) restricted introgression in Dubois ($\beta > 0$) and extreme (high *L. idas* or high *L. melissa*) ancestry frequencies in Jackson Hole. We were especially interested in the last comparison, as the restricted introgression AIMs constitute our best candidates for regions of the genome associated with reproductive isolation. We initially focused on the top 10% of AIMs in each of the categories (e.g., the 116 out of 1164 AIMs with the highest point estimates of α from `bgc`). We conducted randomization tests by permuting parameters across AIMs to test whether and to what extent AIMs in the top 10% for each category coincided more for each comparison than expected by chance. We conducted 10,000 permutations to generate null expectations. The null distribution was used to calculate a P value and x-fold enrichment for each comparison. As an example, an x-fold enrichment of 2.0 would indicate that twice as many AIMs exhibited a pair of patterns (e.g., restricted introgression in Dubois and extreme ancestry frequencies in Jackson Hole) as expected by chance (based on the mean of the null). We repeated these analyses considering the top 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% and 1% of AIMs in each category (i.e., across more and more extreme quantiles), and with only autosomal or only Z-linked AIMs. We also repeated this analysis with only males and with AIMs defined as those SNPs with an allele frequency difference of $\geq$0.2 between *L. melissa* and *L. idas* to further assess the robustness of our results.

Finally, we extracted IPR and GO terms and descriptions generated by `maker` for the set of AIMs in the top 10% for each comparison where we had evidence of significantly greater overlap between categories than expected by chance. We filtered the IPR terms based on their hierarchical classifications (i.e., superfamily, family, domain or repeat). We retained unique terms and dropped terms that at lower or overlapping levels in the Interproscan database [72]. For example, if a SNP was annotated for a superfamily IPR term and multiple domains within the superfamily, we retained only the superfamily term.

Whole genome phylogenomics

We next asked whether patterns of introgression in the contemporary, Dubois hybrid zone were also predictive of patterns of ancestry in two additional, ancient hybrid lineages from the Sierra Nevada and Warner mountains of western North America [6, 29, 25]. To do this, we generated high-coverage, whole genome sequences from *L. anna* (from Yuba Gap, CA), *L. melissa* (from Bonneville Shoreline, UT), *L. idas* (from Trout Lake, WY), and the Sierra Nevada (from Carson Pass, NV), and Warner mountain (from Buck Mountain, CA) lineages. We extracted DNA from one female (ZW) butterfly per population using Qiagen's MagAttract HMW DNA extraction kit (Qiagen, inc.) following the manufacture's suggested protocol. We then outsourced library construction and sequencing to Macrogen Inc. (Seoul, South Korea). One standard paired-end shotgun library (180 bp insert) was constructed for each butterfly/lineage

using a TruSeq library preparation kit (Illumina, Inc.). Each library was sequenced in its own lane on a HiSeq 2000 with 2 $\times$ 100 bp paired reads. We obtained $>$ 20 million high quality ($\geq$ Q30) for each library.

We ran `bwa mem` with a minimum seed length of 15, internal seeds of longer than 20 bp, and only output alignments with a quality score of $\geq$30. We then used `samtools` (version 1.5) to compress, sort and index the alignments, and to remove PCR duplicates [76]. We used GATK's HaplotypeCaller (version 3.5) to call SNPs across the five genomes [80]. We excluded bases with mapping base quality scores less than 30, assumed a prior heterozygosity of 0.001, applied the aggressive PCR indel model, and only called variants with a minimum confidence threshold of 50. We used the intermediate g.vcf file approach followed by joint variant calling with the `GenotypeGVCFs` command when calling variants. We then filtered the initial set of variants to include only SNPs with an average minimum coverage of 6$\times$, maximum absolute values of 2.5 for the base quality rank sum test, the mapping quality rank sum test and the read position rank sum test, a minimum ratio of variant confidence to non-reference read depth of 2.5, and a minimum mapping quality of 30. We also excluded all indels and SNPs with more than two alleles, and all SNPs on smaller scaffolds not assigned to linkage groups. Finally, SNPs with exceptionally high coverage ($>$450$\times$) or that were clustered together (within 5 bps of each other) were dropped. This left us with 2,054,096 SNPs for phylogenomic analyses.

We first estimated a genome consensus or "species" tree from a concatenated alignment of the autosomal SNPs (i.e., this analysis did not include SNPs on the Z chromosome). We estimated the tree with `RAxML` (version 8.2.9) [81] under the GTR model with no rate heterogeneity. 100 bootstrap replicates were generated and analyzed to assess confidence in the bifurcations in the estimated phylogeny. Next, to examine variation in (unrooted) tree topologies across the genome, we split the alignment into non-overlapping 1000 SNP windows. We estimated phylogenies for each window using `RAxML` as described above. We then used the `R` packages `ape` (version 5.2) [82] and `phytools` (version 0.6.60) [83] to identify trees (across windows) with the same topology and to quantify the proportion/number of trees on each linkage group with each topology. Finally, we used randomization tests to determine whether autosomes, the Z chromosome, or the 49 candidate barrier loci (i.e., regions containing the 49 AIMs with restricted introgression in Dubois and extreme ancestry frequencies in Jackson Hole) exhibited a significant excess or deficit of a given topology. This was done in `R` as well. We used 1000 randomizations (permutations) of tree topologies across 1000 SNP windows (and thus linkage groups) for each analysis.

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Data Archiving

DNA sequence data have been archived on NCBIs SRA (accession numbers pending). Computer code and other key input files are available on DRYAD (accession number pending).

References

[1] Arnold, M. L. *Natural hybridization and evolution* (Oxford University Press, 1997).

[2] Mallet, J. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* **20**, 229–237 (2005).

[3] Prüfer, K. *et al.* The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43 (2014).

[4] De Manuel, M. *et al.* Chimpanzee genomic diversity reveals ancient admixture with bonobos. *Science* **354**, 477–481 (2016).

[5] Taylor, S. A. & Larson, E. L. Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. *Nature Ecology & Evolution* **3**, 170 (2019).

[6] Gompert, Z., Fordyce, J. A., Forister, M. L., Shapiro, A. M. & Nice, C. C. Homoploid hybrid speciation in an extreme habitat. *Science* **314**, 1923–1925 (2006).

[7] Buerkle, C. A. & Rieseberg, L. H. The rate of genome stabilization in homoploid hybrid species. *Evolution* **62**, 266–275 (2008).

[8] Sankararaman, S. *et al.* The genomic landscape of Neanderthal ancestry in present-day humans. *Nature* **507**, 354 (2014). URL `http://dx.doi.org/10.1038/nature12961`.

[9] Schumer, M., Cui, R., Powell, D. L., Rosenthal, G. G. & Andolfatto, P. Ancient hybridization and genomic stabilization in a swordtail fish. *Molecular Ecology* **25**, 2661–2679 (2016). URL `http://dx.doi.org/10.1111/mec.13602`.

[10] Elgvin, T. O. *et al.* The genomic mosaicism of hybrid speciation. *Science Advances* **3**, e1602996 (2017). URL `http://dx.doi.org/10.1126/sciadv.1602996`.

[11] Lamichhaney, S. *et al.* Rapid hybrid speciation in Darwin's finches. *Science* **359**, 224–228 (2018).

[12] Barton, N. H. & Hewitt, G. M. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16**, 113–148 (1985).

[13] Abbott, R. J., Barton, N. H. & Good, J. M. Genomics of hybridization and its evolutionary consequences. *Molecular Ecology* **25**, 2325–2332 (2016). URL `http://dx.doi.org/10.1111/mec.13685`.

[14] Gompert, Z., Mandeville, E. G. & Buerkle, C. A. Analysis of population genomic data from hybrid zones. *Annual Review of Ecology, Evolution, and Systematics* **48** (2017).

[15] Gompert, Z. A continuous correlated beta process model for genetic ancestry in admixed populations. *PloS ONE* **11**, e0151047 (2016).

[16] Schumer, M. *et al.* Natural selection interacts with recombination to shape the evolution of hybrid genomes. *Science* **360**, 656–660 (2018).

[17] Barton, N. & Bengtsson, B. O. The barrier to genetic exchange between hybridising populations. *Heredity* **57**, 357 (1986).

[18] Rieseberg, L. H., Whitton, J. & Gardner, K. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* **152**, 713–727 (1999).

[19] Payseur, B. A., Krenz, J. G. & Nachman, M. W. Differential patterns of introgression across the x chromosome in a hybrid zone between two species of house mice. *Evolution* **58**, 2064–2078 (2004).

[20] Harrison, R. G. & Larson, E. L. Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. *Molecular Ecology* **25**, 2454–2466 (2016). URL `http://dx.doi.org/10.1111/mec.13582`.

[21] Payseur, B. A. & Rieseberg, L. H. A genomic perspective on hybridization and speciation. *Molecular Ecology* **25**, 2337–2360 (2016). URL `http://dx.doi.org/10.1111/mec.13557`.

[22] Rieseberg, L. H. *et al.* Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301**, 1211–1216 (2003).

[23] Mandeville, E. G. *et al.* Inconsistent reproductive isolation revealed by interactions between *Catostomus* fish species. *Evolution Letters* **1**, 255–268 (2017).

[24] Forister, M. L., Gompert, Z., Fordyce, J. A. & Nice, C. C. After 60 years, an answer to the question: what is the Karner blue butterfly? *Biology Letters* **7**, 399–402 (2011).

[25] Gompert, Z. *et al.* Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular Ecology* **23**, 4555–4573 (2014). URL `http://dx.doi.org/10.1111/mec.12811`.

[26] Gompert, Z., Lucas, L. K., Fordyce, J. A., Forister, M. L. & Nice, C. C. Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone. *Molecular Ecology* **19**, 3171–3192 (2010). URL `http://dx.doi.org/10.1111/j.1365-294x.2010.04727.x`.

[27] Gompert, Z. *et al.* Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. *Evolution* **66**, 2167–2181 (2012). URL `http://dx.doi.org/10.1111/j.1558-5646.2012.01587.x`.

[28] Gompert, Z. *et al.* Geographically multifarious phenotypic divergence during speciation. *Ecology and Evolution* **3**, 595–613 (2013). URL `http://dx.doi.org/10.1002/ece3.445`.

[29] Nice, C. C. *et al.* Hybrid speciation and independent evolution in lineages of alpine butterflies. *Evolution* **67**, 1055–1068 (2013). URL `http://dx.doi.org/10.1111/evo.12019`.

[30] Gompert, Z. & Buerkle, C. `bgc`: Software for Bayesian estimation of genomic clines. *Molecular Ecology Resources* **12**, 1168–1176 (2012).

[31] Gompert, Z. & Buerkle, C. A. Bayesian estimation of genomic clines. *Molecular Ecology* **20**, 2111–2127 (2011).

[32] Gompert, Z., Parchman, T. L. & Buerkle, C. A. Genomics of isolation in hybrids. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 439–450 (2012).

[33] Stanic, B. *et al.* Cold hardiness in *Ostrinia nubilalis* (Lepidoptera: Pyralidae): Glycerol content, hexose monophosphate shunt activity, and antioxidative defense system. *European Journal of Entomology* **101**, 459–466 (2004).

[34] Fan, L., Lin, J., Zhong, Y. & Liu, J. Shotgun proteomic analysis on the diapause and non-diapause eggs of domesticated silkworm *Bombyx mori*. *PLoS One* **8**, e60386 (2013).

[35] Adrion, J. R., Hahn, M. W. & Cooper, B. S. Revisiting classic clines in *Drosophila melanogaster* in the age of genomics. *Trends in Genetics* **31**, 434–444 (2015).

[36] Hagen, R. & Scriber, J. Sex-linked diapause, color, and allozyme loci in *Papilio glaucus*: linkage analysis and significance in a hybrid zone. *Journal of Heredity* **80**, 179–185 (1989).

[37] Scriber, J. M., Giebink, B. L. & Snider, D. Reciprocal latitudinal clines in oviposition behavior of *Papilio glaucus* and *P. canadensis* across the Great Lakes hybrid zone: possible sex-linkage of oviposition preferences. *Oecologia* **87**, 360–368 (1991).

[38] Cong, Q., Borek, D., Otwinowski, Z. & Grishin, N. V. Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. *Cell Reports* **10**, 910–919 (2015).

[39] Mandrioli, M., Monti, M. & Tedeschi, R. Presence and conservation of the immunoglobulin superfamily in insects: current perspective and future challenges. *Invertebrate Survival Journal* **12**, 188–194 (2015).

[40] Teeter, K. C. *et al.* Genome-wide patterns of gene flow across a house mouse hybrid zone. *Genome Research* **18**, 67–76 (2008).

[41] Ozaki, K., Utoguchi, A., Yamada, A. & Yoshikawa, H. Identification and genomic structure of chemosensory proteins (CSP) and odorant binding proteins (OBP) genes expressed in foreleg tarsi of the swallowtail butterfly *Papilio xuthus*. *Insect Biochemistry and Molecular Biology* **38**, 969–976 (2008).

[42] Briscoe, A. D. *et al.* Female behaviour drives expression and evolution of gustatory receptors in butterflies. *PLoS Genetics* **9**, e1003620 (2013).

[43] Martin, A. *et al.* Diversification of complex butterfly wing patterns by repeated regulatory evolution of a Wnt ligand. *Proceedings of the National Academy of Sciences* **109**, 12632–12637 (2012).

[44] Gompert, Z., Fordyce, J. A., Forister, M. L. & Nice, C. C. Recent colonization and radiation of North American *Lycaeides* (*Plebejus*) inferred from mtDNA. *Molecular Phylogenetics and Evolution* **48**, 481–490 (2008).

[45] Gould, N. E.-S. J. & Eldredge, N. Punctuated equilibria: an alternative to phyletic gradualism. *Essential Readings in Evolutionary Biology* 82–115 (1972).

[46] Reznick, D. N. & Ricklefs, R. E. Darwin's bridge between microevolution and macroevolution. *Nature* **457**, 837 (2009).

[47] Wagner, C. E., Harmon, L. J. & Seehausen, O. Ecological opportunity and sexual selection together predict adaptive radiation. *Nature* **487**, 366 (2012).

[48] Rieseberg, L. H., Sinervo, B., Linder, C. R., Ungerer, M. C. & Arias, D. M. Role of gene interactions in hybrid speciation: Evidence from ancient and experimental hybrids. *Science* **272**, 741–745 (1996). URL `http://dx.doi.org/10.1126/science.272.5262.741`.

[49] DiFazio, S. P., Slavov, G. T., Joshi, C. P. *et al.* *Populus*: a premier pioneer system for plant genomics. *Genetics, genomics and breeding of poplar. Enfield, NH: Science Publishers* 1–28 (2011).

[50] Baute, G. J., Owens, G. L., Bock, D. G. & Rieseberg, L. H. Genome-wide genotyping-by-sequencing data provide a high-resolution view of wild *Helianthus* diversity, genetic structure, and interspecies gene flow. *American Journal of Botany* **103**, 2170–2177 (2016).

[51] Chhatre, V. E., Evans, L. M., DiFazio, S. P. & Keller, S. R. Adaptive introgression and maintenance of a trispecies hybrid complex in range-edge populations of *Populus*. *Molecular Ecology* **27**, 4820–4838 (2018).

[52] Endler, J. A. *Natural selection in the wild.* 21 (Princeton University Press, 1986).

[53] Soria-Carrasco, V. *et al.* Stick insect genomes reveal natural selection's role in parallel speciation. *Science* **344**, 738–742 (2014).

[54] Masly, J. P. & Presgraves, D. C. High-resolution genome-wide dissection of the two rules of speciation in *Drosophila*. *PLoS Biology* **5**, e243 (2007).

[55] Coyne, J. A. & Allen, O. H. *Speciation* (Sinauer Associates, Inc., 2004).

[56] Counterman, B. A. *et al.* Genomic hotspots for adaptation: the population genetics of Müllerian mimicry in *Heliconius erato*. *PLoS Genetics* **6**, e1000796 (2010).

[57] Martin, A. & Orgogozo, V. The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution* **67**, 1235–1250 (2013).

[58] Gompert, Z. *et al.* The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Molecular Ecology* **24**, 2777–2793 (2015).

[59] Chaturvedi, S. *et al.* The predictability of genomic changes underlying a recent host shift in Melissa blue butterflies. *Molecular Ecology* **27**, 2651–2666 (2018).

[60] Mifsud, B. *et al.* Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. *Nature Genetics* **47**, 598 (2015).

[61] Putnam, N. H. *et al.* Chromosome-scale shotgun assembly using an in vitro method for long-range linkage. *Genome Research* **26**, 342–350 (2016).

[62] Delcher, A. L., Phillippy, A., Carlton, J. & Salzberg, S. L. Fast algorithms for large-scale genome alignment and comparison. *Nucleic Acids Research* **30**, 2478–2483 (2002).

[63] Yandell, M. & Ence, D. A beginner's guide to eukaryotic genome annotation. *Nature Reviews Genetics* **13**, 329 (2012).

[64] Campbell, M. S., Holt, C., Moore, B. & Yandell, M. Genome annotation and curation using maker and maker-p. *Current Protocols in Bioinformatics* **48**, 4–11 (2014).

[65] Price, A. L., Jones, N. C. & Pevzner, P. A. *De novo* identification of repeat families in large genomes. *Bioinformatics* **21**, i351–i358 (2005).

[66] Challis, R. J., Kumar, S., Dasmahapatra, K. K. K., Jiggins, C. D. & Blaxter, M. Lepbase: the lepidopteran genome database. *BioRxiv* 056994 (2016).

[67] Grabherr, M. G. *et al.* Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**, 644 (2011).

[68] Haas, B. J. *et al.* De novo transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. *Nature Protocols* **8**, 1494 (2013).

[69] Korf, I. Gene finding in novel genomes. *BMC Bioinformatics* **5**, 59 (2004).

[70] Stanke, M. & Morgenstern, B. Augustus: a web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Research* **33**, W465–W467 (2005).

[71] UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research* **47**, D506–D515 (2018).

[72] Jones, P. *et al.* InterProScan 5: genome-scale protein function classification. *Bioinformatics* **30**, 1236–1240 (2014).

[73] Gompert, Z. *et al.* Experimental evidence for ecological selection on genome variation in the wild. *Ecology Letters* **17**, 369–379 (2014).

[74] Li, H. & Durbin, R. Fast and accurate short read alignment with burrows–wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).

[75] Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv preprint arXiv:1303.3997* (2013).

[76] Li, H. *et al.* The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).

[77] Pritchard, J. K., Stephens, M. & Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959 (2000).

[78] Li, H. A statistical framework for snp calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27**, 2987–2993 (2011).

[79] Szymura, J. M. & Barton, N. H. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. *Evolution* **40**, 1141–1159 (1986).

[80] McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* **20**, 1297–1303 (2010).

[81] Stamatakis, A. `RAxML` version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).

[82] Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2018).

[83] Revell, L. J. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**, 217–223 (2012).

Figures

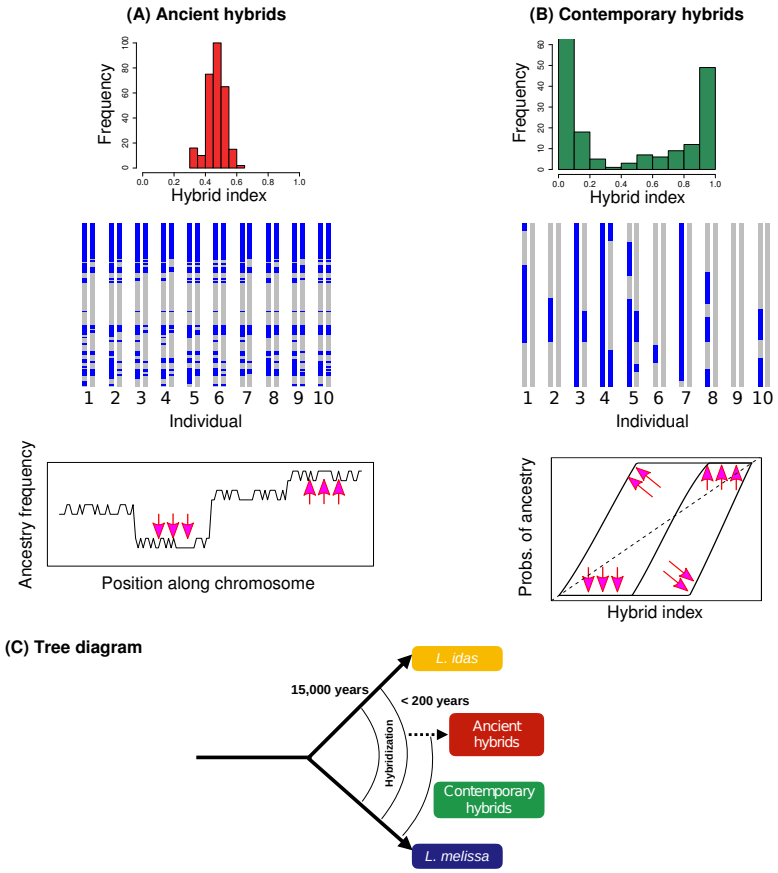

Figure 1: Conceptual overview and a comparative summary of genomic patterns expected in ancient hybrids (A) versus contemporary hybrids (B). Histograms show narrow (ancient) versus wide (contemporary) distributions of hybrid indexes. In ancient hybrids, ancestry blocks (gray versus blue segments) have been broken up by recombination and some have stabilized, that is fixed within the hybrid lineage. In contemporary hybrids, larger ancestry blocks are expected and these vary more among individuals. Plots show the expected effect of selection on ancestry frequencies and patterns of introgression in ancient and contemporary hybrids, respectively. In ancient hybrids, selection (pink arrows) shifts ancestry frequencies. In contemporary hybrids, selection shifts genomic clines for individual loci relative to the genome-wide average (dashed line). (C) Diagram represents history of hybridization in *Lycaeides*.

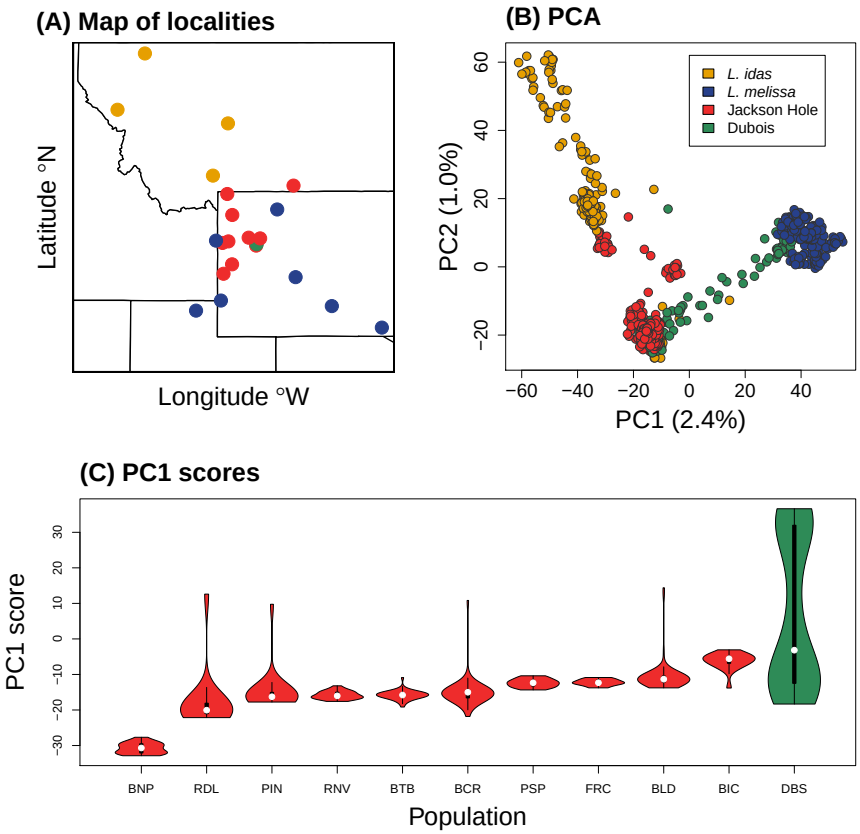

Figure 2: (A) Map of sample locations with points colored based on nominal taxa (Table S1). (B) Ordination of genetic variation via principal component analysis (PCA). Points denote indiviudals (a few low-coverage individuals were removed for visualization). (C) Violin plots show variation in genome composition within and among 10 Jackson Hole *Lycaeides* populations and the Dubois hybrid zone (DBS) based on PC1 scores.

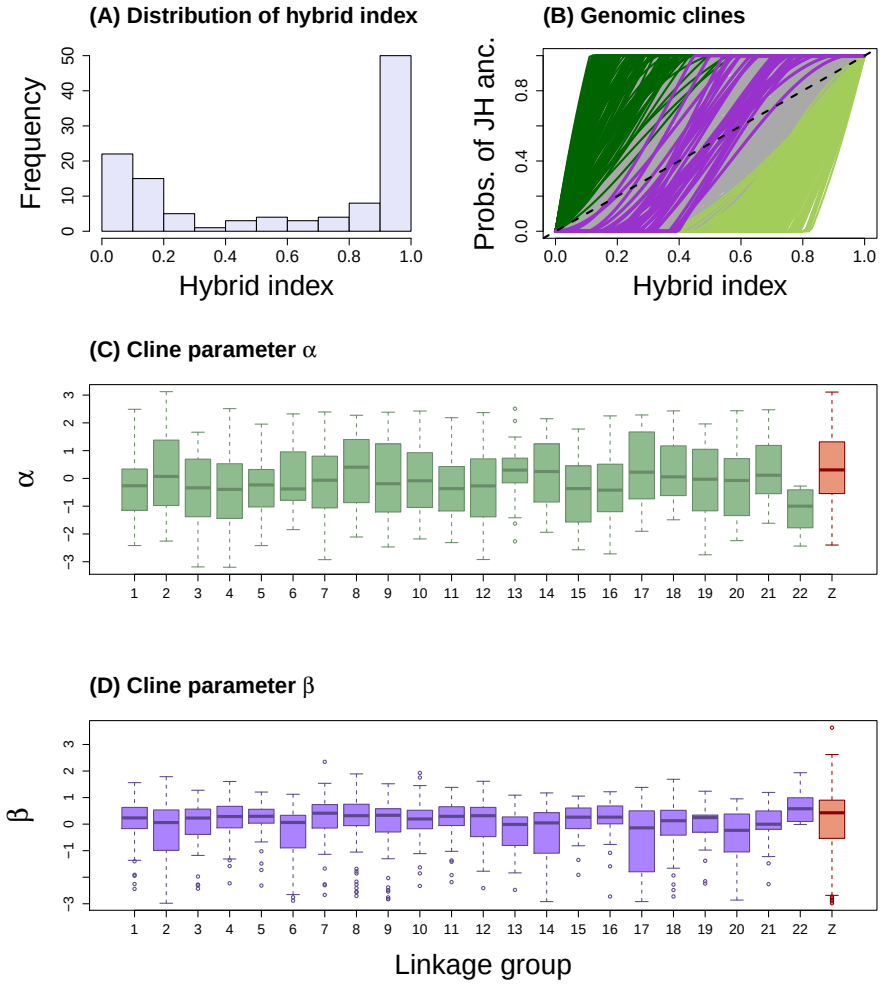

Figure 3: Summary of the genomic clines analysis. (A) The histogram depicts the distribution of hybrid indices in the Dubois hybrid zone. (B) This plot shows estimated genomic clines for a subset of ancestry informative SNPs (AIMs). Each solid line gives the estimated probability of Jackson Hole (JH) ancestry for an AIM. Green lines denote cases of credible directional introgression (95% CIs for α that exclude zero) and purple lines denote credible cases of restricted introgression (95% CIs for $\beta > 0$) (gray lines denote clines not credibly different from the genome-average). The dashed line gives the null expectation based on genome-wide admixture. Boxplots show the distribution of cline paramters α (C) and β (D) across loci for each linkage group.

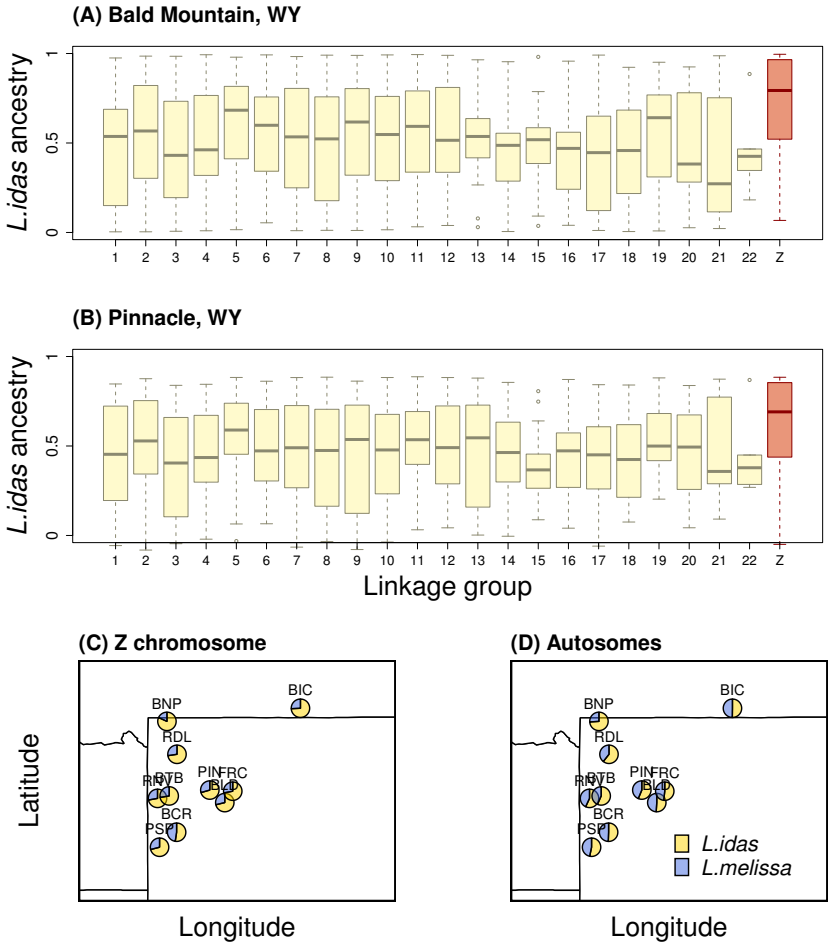

Figure 4: Boxplots show the distribution of *L. idas* ancestry across ancestry informative SNPs (AIMs) for each linkage group in two representative Jackson Hole *Lycaeides* populations–Bald Mountain, WY (BLD) (A) and Pinnacle, WY (PIN) (B). See Fig. S11 for additional populations. The Z sex chromosome is shown in red. Panels (C) and (D) show maps with pie charts reflecting the proportion of *L. idas* and *L. melissa* ancestry (mean) for the Z chromosome (C) and autosomes (D) for each of the ten populations (see Table S1 for population IDs).

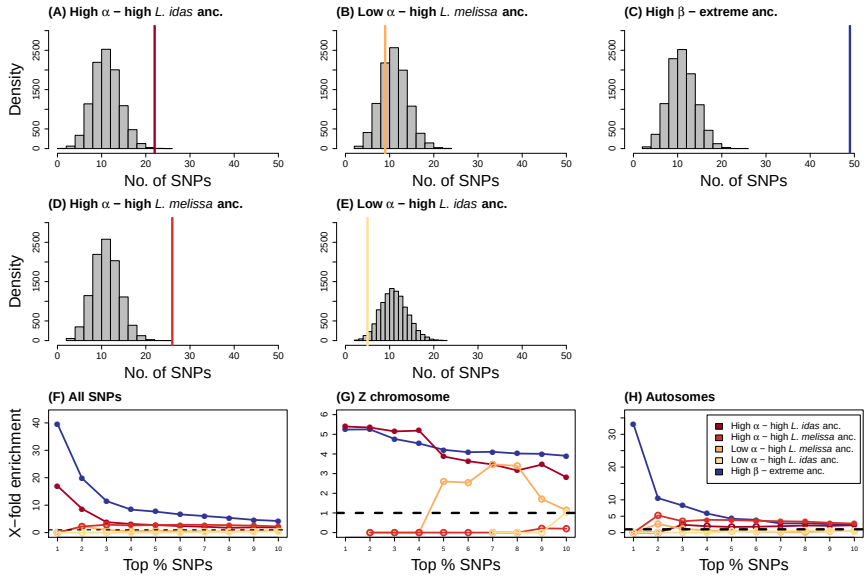

Figure 5: Expected and observed numbers of SNPs with exceptional patterns of introgression in the Dubois hybrid zone and extreme ancestry frequencies in Jackson Hole *Lycaedes*. Panels A-E show results when considering the top 10% of AIMs in each category. Histograms give null expectations from randomization tests and vertical solid lines show the observed number of AIMs exhibiting a given pattern. Comparisons shown are: directional introgression of Jackson Hole alleles (high α) and high *L. idas* ancestry (in Jackson Hole) (A), directional introgression of *L. melissa* alleles (low α) and high *L. melissa* ancestry (B), restricted introgression (high β) and extremely high *L. idas* or *L. melissa* ancestry (C), directional introgression of Jackson Hole alleles (high α) and high *L. melissa* ancestry (B), and directional introgression of *L. melissa* alleles (low α) and high *L. idas* ancestry (E). Panels F-H show how these results are affected by considering different levels of stringency (i.e., by examining the most extreme 10% to the top 1% of AIMs with each pattern), and when considering only the Z chromosome (G) or only the autosomes (H). Here, circles denote the ratio of the observed to expected overlap from the null, and the circles are filled ($p \leq 0.05$) or not ($p > 0.05$) to denote whether the overlap is greater than expected by chance.

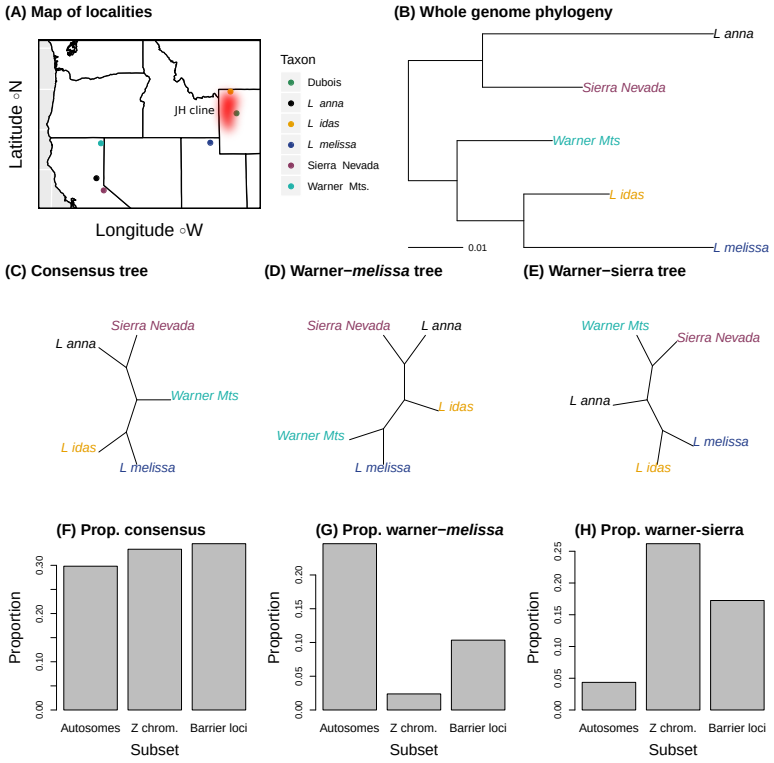

Figure 6: Whole-genome phylogenetic analyses. Panel (A) shows a map of the sampling localities in the western USA; Dubois is included for reference but was not sampled for whole-genome phylogenetics. The Jackson Hole ancestry cline (i.e., the range of the ancient Jackson Hole hybrids) is shown for reference as well (red zone). Panel B shows the midpoint rooted maximum likelihood phylogram inferred from the whole-genome SNP set (2,013,201 autosomal SNPs). Panels (C), (D) and (E) show three common unrooted tree topologies inferred from 1000 SNP windows. The tree in panel (C) is the most common topology and matches the whole-genome tree in (B). Trees in panels (D) and (E) differ by grouping the Warner Mts. population with *L. melissa* or the Sierra Nevada population, respectively. Panels (F), (G), and (H) show the proportion of 1000 SNP topologies matching the trees in (C)–(E) for autosomal windows, Z chromosome windows, and the 1000 SNP windows that contain the candidate barrier loci (see main text). There is a significant deficit of the topology in (D) on the Z chromosome and among the barrier loci (x-fold = 0.10, $P < 0.001$ and x-fold = 0.43, $P = 0.047$, respectively), and a significant excess of topology (E) on the Z chromosome and among the barrier loci (x-fold = 5.36, $P < 0.001$ and x-fold = 3.62, $P = 0.008$).

Supplemental Tables and Figures

Table S1: Locality information and sample sizes (N) for the populations included in this study. * denote new sequence data generated for this study, other sequence data were previously presented in [25].

Locality	**ID**	**Taxon**	**Longitude**	**Latitude**	**N**
Bonneville, UT	BST	*L. melissa*	-111.7939	41.7250	*24
Cody, WY	CDY	*L. melissa*	-108.9780	44.5121	23
Cokeville, WY	CKV	*L. melissa*	-110.9373	42.0052	10
Lander, WY	LAN	*L. melissa*	-108.3551	42.6533	24
Montague, CA	MON	*L. melissa*	-107.8162	38.3746	20
Yellow Pine, WY	YWP	*L. melissa*	-105.4006	41.2519	20
Sinclair, WY	SIN	*L. melissa*	-107.1131	41.8511	97
Victor, ID	VIC	*L. melissa*	-111.1114	43.6590	20
Garnet Peak, MT	GNP	*L. idas*	-111.2245	45.4323	98
King's Hill, MT	KHL	*L. idas*	-110.6990	46.8407	18
Soldier Creek, MT	SDC	*L. idas*	-114.6119	47.2062	20
Siyeh Creek, MT	SYV	*L. idas*	-113.6681	48.7026	20
Bald Mountain, WY	BLD	*L. idas*	-109.7161	43.5225	*74
Bull Creek, WY	BCR	Jackson Hole	-110.5530	43.0076	46
Big Ice Cave, WY	BIC	Jackson Hole	-108.4012	45.1617	18
Bunsen Peak, WY	BNP	Jackson Hole	-110.7212	44.9337	20
Blacktail Butte, WY	BTB	Jackson Hole	-110.6820	43.6382	46
Frontier Creek, WY	FRC	Jackson Hole	-109.5776	43.7206	*20
Pinnacle, WY	PIN	Jackson Hole	-109.9762	43.7401	20
Periodic Springs, WY	PSP	Jackson Hole	-110.8493	42.7468	20
Riddle Lake, WY	RDL	Jackson Hole	-110.5465	44.3617	30
Rendevouz Mtn., WY	RNV	Jackson Hole	-110.8847	43.5957	32
Dubois, WY	DBS	Hybrid zone	-109.6991	43.56229	*115

Table S2: Summary of the number of AIMs with credible evidence of excess directional introgression of Jackson Hole *Lycaeides* alleles in the Dubois hybrid zone ($\alpha > 0$) in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	79	1.10	0.13
near gene	93	1.10	0.10
on cds	29	1.08	0.36
near cds	78	1.07	0.21
on TE	5	0.94	0.64
near TE	24	1.05	0.44
on protein	98	1.04	0.30
near protein	124	1.04	0.23

Table S3: Summary of the number of AIMs with credible evidence of excess directional introgression of *L. melissa* alleles in the Dubois hybrid zone ($\alpha < 0$) in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	112	1.08	0.13
near gene	129	1.06	0.19
on CDS	53	1.36	**< 0.01**
near CDS	116	1.11	0.06
on TE	9	1.16	0.36
near TE	36	1.09	0.30
on protein	155	1.14	**0.01**
near protein	185	1.08	**0.03**

Table S4: Summary of the number of AIMs with credible evidence of restricted introgression in the Dubois hybrid zone ($\beta > 0$) in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	14	1.12	0.36
near gene	14	0.95	0.68
on CDS	5	1.06	0.51
near CDS	14	1.10	0.38
on TE	3	3.16	0.06
near TE	3	0.75	0.79
on protein	16	0.97	0.63
near protein	19	0.91	0.80

Table S5: Summary of the observed and expected number of AIMs with the highest *L. idas* ancestry frequencies in Jackson Hole *Lycaeides* (top 10%) that were on the Z sex chromosome. Results are shown for each Jackson Hole population. We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Locality	**No. observed**	**x-fold enrichment**	***P***
BCR	29	1.28	0.08
BIC	75	3.32	**< 0.01**
BLD	66	2.92	**< 0.01**
BNP	66	2.93	**< 0.01**
BTB	58	2.57	**< 0.01**
FRC	65	2.86	**< 0.01**
PIN	64	2.83	**< 0.01**
PSP	70	3.11	**< 0.01**
RDL	52	2.30	**< 0.01**
RNV	60	2.65	**< 0.01**

Table S6: Table shows summary of randomization tests for presence of top 10% SNPs showing excess *L. melissa* ancestry frequency on Z chromosome for 10 Jackson Hole-*Lycaeides* localities (x-fold = Number of SNPs observed is how much more than chance; P = randomization-based P-values for the null hypothesis that the proportion of top SNPs observed is not greater than the genomic proportion). $P \leq 0.05$ are in bold.

Locality	**No. observed**	**x-fold enrichment**	***P***
BCR	36	1.59	**<0.01**
BIC	9	0.39	0.99
BLD	8	0.35	1.00
BNP	18	0.79	0.90
BTB	13	0.57	0.99
FRC	6	0.26	1.00
PIN	12	0.53	0.99
PSP	12	0.52	0.98
RDL	15	0.66	0.98
RNV	15	0.65	0.99

Table S7: Summary of the number of AIMs with the highest *L. idas* ancestry frequencies in Jackson Hole *Lycaeides* (top 10%) in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	53	1.19	0.05
near gene	63	1.20	**0.02**
on CDS	23	1.38	0.06
near CDS	55	1.22	**0.02**
on TE	3	0.90	0.66
near TE	13	0.91	0.67
on protein	61	1.04	0.35
near protein	84	1.14	**0.02**

Table S8: Summary of the number of AIMs with the highest *L. melissa* ancestry frequencies in Jackson Hole *Lycaeides* (top 10%) in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	$\boldsymbol{P}$
on gene	55	1.24	**0.02**
near gene	61	1.17	0.05
on CDS	18	1.08	0.39
near CDS	52	1.16	0.09
on TE	3	0.90	0.66
near TE	9	0.63	0.96
on protein	70	1.20	**0.01**
near protein	79	1.07	0.16

Table S9: Summary of the number of AIMs with restricted introgression in the Dubois hybrid zone (10% with the highest estimates of β from `bgc`) and with extreme (high *L. idas* or *L. melissa*) ancestry frequencies in Jackson Hole (top 10%) that were in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	$\boldsymbol{P}$
on gene	23	1.24	0.12
near gene	27	1.23	0.09
on CDS	8	1.14	0.40
near CDS	22	1.16	0.22
on TE	3	2.17	0.16
near TE	6	1.03	0.55
on protein	24	0.98	0.61
near protein	32	1.04	0.43

Table S10: Summary of the number of AIMs with directional introgression of Jackson Hole alleles in the Dubois hybrid zone (10% with the highest estimates of α from `bgc`) and with high *L. idas* ancestry frequencies in Jackson Hole (top 10%) that were in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	10	1.19	0.30
near gene	13	1.31	0.13
on CDS	5	1.59	0.19
near CDS	12	1.47	0.09
on TE	0	0	0.16
near TE	2	0.77	0.77
on protein	12	1.09	0.41
near protein	16	1.16	0.23

Table S11: Summary of the number of AIMs with directional introgression of Jackson Hole alleles in the Dubois hybrid zone (10% with the highest estimates of α from `bgc`) and with high *L. melissa* ancestry frequencies in Jackson Hole (top 10%) that were in or near (with 1000 bp) genes (this includes all of the sequence elements necessary to encode a functional transcript), coding sequences (including only sequences between start and stop codons; CDS), transposable elements (TEs) and proteins (matches against protein sequences). We report the observed number, and x-fold enrichment and P value from randomization tests. Values of $P \leq 0.05$ are in bold font.

Category	**No. observed**	**x-fold enrichment**	***P***
on gene	13	1.31	0.15
near gene	14	1.20	0.22
on CDS	6	1.63	0.15
near CDS	14	1.40	0.07
on TE	1	1.35	0.53
near TE	2	0.63	0.85
on protein	14	1.07	0.42
near protein	16	0.97	0.64

Table S12: Summary of protein descriptions from `interproscan` for AIMs which had high *L. idas* or *L. melissa* ancestry in the ancient, Jackson Hole hybrids and restricted introgression in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the IPR number and associated description from `interproscan`.

LG	Scaffold	SNPs	Start	Stop	IPR #	Description
11	4	12406546	12403487	12420293	IPR036388	Winged helix-like DNA-binding domain superfamily
					IPR037241	E2F-DP heterodimerization region
2	11	8117866	8115532	8118472	IPR004117	Olfactory receptor, insect
12	833	6668579	6667703	6667757	NA	NA
1	1628	11727258	11720849	11726566	IPR007231	Nucleoporin interacting component Nup93/Nic96
1	1628	12893859	12894703	12894888	NA	NA
23	1631	1090637	1091079	1091110	NA	NA
23	1631	4238467	4237518	4238564	IPR011011	Zinc finger, FYVE/PHD-type
23	1631	5365561	5365704	5366438	NA	NA
23	1631	5516528	5488340	5561251	IPR027417	P-loop containing nucleoside triphosphate hydrolase
23	1631	6146721	6146944	6146977	NA	NA
23	1631	7534776	7528709	7535929	NA	NA
23	1631	7688497	7684329	7697467	IPR011989	Armadillo-like helical
					IPR027651	FH1/FH2 domain-containing protein 3
23	1631	8161313	8155537	8174517	IPR036865	CRAL-TRIO lipid binding domain superfamily
					IPR008936	Rho GTPase activation protein
23	1631	9616373	9611236	9626847	IPR036179	Immunoglobulin-like domain superfamily
23	1631	10080466, 10096614, 10096721	10057941	10097025	IPR036179	Immunoglobulin-like domain superfamily
23	1631	12342151	12341301	12341547	NA	NA
23	1631	12682671	12681749	12681828	NA	NA
23	1631	13728571	13729333	13729356	NA	NA
23	1631	13908210	13908744	13908776	NA	NA
23	1631	13944328	13943280	13943458	NA	NA
23	1631	14175992	14174137	14174993	NA	NA
		14799474			NA	NA
23	1631	15003188	15002491	15002528	NA	NA
23	1631	15044549, 15347619	15044399	15057269	NA	NA
					IPR002017	Spectrin repeat
					IPR011993	PH-like domain superfamily
					IPR035899	Dbl homology (DH) domain superfamily
23	1631	15428089, 15428102	15418766	15469764	NA	NA
23	1631	15630833	15628588	15650639	IPR008927	6-phosphogluconate dehydrogenase-like, C-terminal domain superfamily
					IPR036291	NAD(P)-binding domain superfamily
23	1631	16563238, 16563245	16562585	16571768	NA	NA
23	1631	16742878	16742257	16742291	NA	NA
23	1631	17005201	16989784	17005380	NA	NA
23	1631	17266869	17267147	17267186	NA	NA
23	1631	18504663	18503673	18503704	NA	NA
23	1631	20910914	20911725	20925281	IPR039170	5'-AMP-activated protein kinase subunit gamma-2
23	1631	21035262, 21035340	21034582	21034639	NA	NA
23	1631	21250737, 21250758	21250378	21250415	NA	NA
23	1631	21303464	21296234	21303124	IPR011009	Protein kinase-like domain superfamily
10	1639	8438185	8435143	8440922	IPR023102	Fatty acid synthase, domain 2
10	1639	10026163	10023889	10032998	IPR000648	Oxysterol-binding protein
					IPR011993	PH-like domain superfamily
9	1641	9383455	9371915	9383193	IPR004878	Otopetrin
7	1642	9017721	9012690	9023798	IPR001781	Zinc finger, LIM-type
3	1646	12073166, 12073190	12053895	12075705	IPR011989	Armadillo-like helical
					IPR026818	Adenomatous polyposis coli (APC) family
					IPR011989	Armadillo-like helical
					IPR026818	Adenomatous polyposis coli (APC) family

Table S13: Summary of gene ontology (GO) terms (i.e., classifications) for AIMs which had high *L. idas* or *L. melissa* ancestry in the ancient, Jackson Hole hybrids and restricted introgression in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the GO term numbers (IDs) and descriptions. Symbols denote whether each term corresponds to a biological process (-b), molecular function (-m), or cellular component (-c).

LG	Scaffold	SNPs	Start	Stop	GO #	Description
11	4	12406546	12403487	12420293	GO:0003700-m	DNA-binding transcription factor activity
					GO:0005667-c	Transcription factor complex
					GO:0006355-b	Regulation of transcription, DNA-templated
					GO:0046983-m	Protein dimerization activity
2	11	8117866	8115532	8118472	GO:0004984-m	Olfactory receptor activity
					GO:0005549-m	Odorant binding
					GO:0007608-b	Sensory perception of smell
					GO:0016020-c	Membrane
12	833	6668579	6667703	6667757	NA	NA
1	1628	11727258	11720849	11726566	GO:0005643-c	Nuclear pore
					GO:0017056-m	Structural constituent of nuclear pore
1	1628	12893859	12894703	12894888	NA	NA
23	1631	1090637	1091079	1091110	NA	NA
23	1631	4238467	4237518	4238564	NA	NA
23	1631	5365561	5365704	5366438	NA	NA
23	1631	5516528	5488340	5561251	GO:0003777-m	Microtubule motor activity
					GO:0005524-m	ATP binding
					GO:0005858-c	Axonemal dynein complex
					GO:0007018-b	Microtubule-based movement
					GO:0016887-m	ATPase activity
					GO:0060285-b	Cilium-dependent cell motility
23	1631	6146721	6146944	6146977	NA	NA
23	1631	7534776	7528709	7535929	NA	NA
23	1631	7688497	7684329	7697467	GO:0007015-b	Actin filament organization
23	1631	8161313	8155537	8174517	GO:0007165-b	Signal transduction
23	1631	9616373	9611236	9626847	NA	NA
23	1631	10080466, 10096614, 10096721	10057941	10097025	NA	NA
23	1631	12342151	12341301	12341547	NA	NA
23	1631	12682671	12681749	12681828	NA	NA
23	1631	13728571	13729333	13729356	NA	NA
23	1631	13908210	13908744	13908776	NA	NA
23	1631	13944328	13943280	13943458	NA	NA
23	1631	14175992	14174137	14174993	NA	NA
23	1631	14799474	14799137	14799168	NA	NA
23	1631	15003188	15002491	15002528	NA	NA
23	1631	15044549	15044399	15057269	NA	NA
23	1631	15347619	15326999	15377180	GO:0005089-m	Rho guanyl-nucleotide exchange factor activity
					GO:0005515-m	Protein binding
					GO:0035023-b	Regulation of Rho protein signal transduction
23	1631	15428089, 15428102	15418766	15469764	NA	NA
23	1631	15630833	15628588	15650639	GO:0004616-m	Phosphogluconate dehydrogenase (decarboxylating) activity
					GO:0006098-b	Pentose-phosphate shunt
					GO:0016491-m	Oxidoreductase activity
					GO:0050661-m	NADP binding
					GO:0055114-b	Oxidation-reduction process
23	1631	16563238, 16563245	16562585	16571768	NA	NA
23	1631	16742878	16742257	16742291	NA	NA
23	1631	17005201	16989784	17005380	NA	NA
23	1631	17266869	17267147	17267186	NA	NA
23	1631	18504663	18503673	18503704	NA	NA
23	1631	20910914	20911725	20925281	GO:0032559-m	Regulation of protein serine/threonine kinase activity
23	1631	21035262 21035340	21034582	21034639	NA	NA
23	1631	21250737 21250758	21250378	21250415	NA	NA
23	1631	21303464	21296234	21303124	GO:0004672-m	Protein kinase activity
					GO:0005524-m	ATP binding
					GO:0006468-b	Protein phosphorylation
10	1639	8438185	8435143	8440922	GO:0004312-m	Fatty acid synthase activity
					GO:0009058-b	Biosynthetic process
					GO:0016788-m	Hydrolase activity, acting on ester bonds
10	1639	10026163	10023889	10032998	NA	NA
9	1641	9383455	9371915	9383193	NA	NA
7	1642	9017721	9012690	9023798	NA	NA
3	1646	12073166, 12073190	12053895	12075705	GO:0005515-m	Protein binding
					GO:0008013-m	Beta-catenin binding
					GO:0016055-b	Wnt signaling pathway
					GO:0030178-b	Negative regulation of Wnt signaling pathway

Table S14: Summary of protein descriptions from `interproscan` for AIMs which had high *L. idas* ancestry in the ancient, Jackson Hole hybrids and directional Jackson Hole *Lycaeides* introgression ($\alpha > 0$) in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the IPR number and associated description from `interproscan`.

LG	**Scaffold**	**SNPs**	**Start**	**Stop**	**IPR #**	**Description**
2	11	6234637	6215867	6240330	IPR001930	Peptidase M1, alanine aminopeptidase/leukotriene A4 hydrolase
					IPR024571	ERAP1-like C-terminal domain
2	11	15712950	15710210	15713015	IPR009851	Modifier of rudimentary, Modr
2	11	15712950	NA	NA	IPR037859	Vacuolar protein sorting-associated protein 37
16	503	6014039	NA	NA	NA	NA
12	833	6668579	6667703	6667757	NA	NA
23	1631	1200324	1197667	1204058	IPR036719	Neurotransmitter-gated ion-channel transmembrane domain superfamily
23	1631	1200324	NA	NA	IPR036734	Neurotransmitter-gated ion-channel ligand-binding domain superfamily
23	1631	2570345	2570129	2570178	NA	NA
23	1631	4238526	4237518	4238564	IPR011011	Zinc finger, FYVE/PHD-type
23	1631	9958857	9955713	9968188	IPR027266	GTP-binding protein TrmE/Glycine cleavage system T protein, domain 1
23	1631	9958857	NA	NA	IPR036188	FAD/NAD(P)-binding domain superfamily
23	1631	10080421	10057941	10097025	IPR036179	Immunoglobulin-like domain superfamily
23	1631	10080466	10057941	10097025	IPR036179	Immunoglobulin-like domain superfamily
23	1631	12954191	12954213	12954434	NA	NA
23	1631	14218357	14216920	14217629	NA	NA
23	1631	14760795	14746596	14779181	IPR000034	Laminin IV
					IPR002049	Laminin EGF domain
					IPR036055	LDL receptor-like superfamily
					IPR036179	Immunoglobulin-like domain superfamily
23	1631	15408704	15409126	15409167	NA	NA
23	1631	15428179	15418766	15469764	NA	NA
23	1631	20910914	20911725	20925281	IPR000644	CBS domain
					IPR039170	5'-AMP-activated protein kinase subunit gamma-2
13	1632	6146628	6145294	6150912	IPR011047	Quinoprotein alcohol dehydrogenase-like superfamily
10	1639	13668785	13668901	13668943	NA	NA
9	1641	9384153	9371915	9383193	IPR004878	Otopetrin
7	1642	9023853	9012690	9025104	IPR001781	Zinc finger, LIM-type
4	1648	13426790	13426596	13426775	NA	NA
4	1648	13619133	13600937	13619564	IPR000076	K/Cl co-transporter

Table S15: Summary of gene ontology (GO) terms (i.e., classifications) for AIMs which had high *L. idas* ancestry in the ancient, Jackson Hole hybrids and directional Jackson Hole *Lycaeides* introgression ($\alpha > 0$) in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the GO term numbers (IDs) and descriptions. Symbols denote whether each term corresponds to a biological process (-b), molecular function (-m), or cellular component (-c).

LG	**Scaffold**	**SNPs**	**Start**	**Stop**	**GO #**	**Description**
2	11	6234637	6234637	6215867	GO:0006508-b	Proteolysis
					GO:0008237-m	Metallopeptidase activity
					GO:0008270-m	Zinc ion binding
2	11	15712950	15710210	15713015	GO:0000813-c	ESCRT I complex
					GO:0032509-b	Endosome transport via multivesicular body sorting pathway
16	503	6014039	NA	NA	NA	NA
12	833	6668579	6667703	6667757	NA	NA
23	1631	1200324	1197667	1204058	GO:0004888-m	Transmembrane signaling receptor activity
					GO:0004890-m	GABA-A receptor activity
					GO:0005216-m	Ion channel activity
					GO:0005230-m	Extracellular ligand-gated ion channel activity
					GO:0006811-b	Ion transport
					GO:0016020-c	Membrane
					GO:0016021-c	Integral component of membrane
					GO:0034220-b	Ion transmembrane transport
23	1631	2570345	2570129	2570178	NA	NA
23	1631	4238526	4237518	4238564	NA	NA
23	1631	9958857	9955713	9968188	GO:0005515-m	Protein binding
					GO:0016491-m	Oxidoreductase activity
					GO:0055114-b	Oxidation-reduction process
23	1631	10080421, 10080466	10057941	10097025	NA	NA
23	1631	12954191	12954213	12954434	NA	NA
23	1631	14218357	14216920	14217629	NA	NA
23	1631	14760795	14746596	14779181	GO:0005515-m	Protein binding
23	1631	15408704	15409126	15409167	NA	NA
23	1631	15428179	15418766	15469764	NA	NA
23	1631	20910914	20911725	20925281	GO:0032559-m	Adenyl ribonucleotide binding
					GO:0071900-b	Regulation of protein serine/threonine kinase activity
13	1632	6146628	6146628	6145294	NA	NA
10	1639	13668785	13668785	13668901	NA	NA
9	1641	9384153	9384153	9371915	NA	NA
7	1642	9023853	9023853	9012690	NA	NA
4	1648	13426790	13426790	13426596	NA	NA
4	1648	13619133	13619133	13600937	GO:0005215-m	Transporter activity
					GO:0005887-c	Integral component of plasma membrane
					GO:0006811-b	Ion transport
					GO:0015379-m	Potassium:chloride symporter activity
					GO:0016020-c	Membrane
					GO:0016021-c	Integral component of membrane
					GO:0055085-b	Transmembrane transport
					GO:0071477-b	Cellular hypotonic salinity response
					GO:1902476-b	Chloride transmembrane transport

Table S16: Summary of protein descriptions from `interproscan` for AIMs which had high *L. melissa* ancestry in the ancient, Jackson Hole hybrids and directional Jackson Hole *Lycaeides* introgression ($\alpha > 0$) in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the IPR number and associated description from `interproscan`.

LG	**Scaffold**	**SNPs**	**Start**	**Stop**	**IPR #**	**Description**
11	4	4190884	4190808	4190860	NA	NA
2	11	7335097	7335985	7336153	NA	NA
2	11	8691829	8691651	8691894	NA	NA
2	11	10860558,	10855256	10861383	IPR013083	Zinc finger
		10860564			IPR034732	RING/FYVE/PHD-type
17	309	8389080,	8389311	8389348	NA	NA
		8389096			NA	NA
17	309	12340022	12331408	12343477	IPR004210	BESS motif
					IPR006578	MADF domain
	758	2023	NA	NA	NA	NA
21	1095	7019482,	7019198	7025723	IPR039767	RalA-binding protein 1
		7019493,				
		7019553				
1	1628	2792953	2782773	2797741	IPR004274	FCP1 homology domain
1	1628	13677379	NA	NA	NA	NA
23	1631	6116900	6109396	6117970	IPR000159	Ras-associating (RA) domain,Pleckstrin homology domain
10	1639	12335336	12333398	12336053	IPR029526	PiggyBac transposable element-derived protein
9	1641	13685914	13678341	13685134	IPR006621	Nose resistant-to-fluoxetine protein, N-terminal
7	1642	11407787	11392125	11409041	IPR011701	Major facilitator superfamily
8	1645	9366622	9365131	9365852	NA	NA
8	1645	9587985	9587772	9588024	NA	NA
8	1645	15122056	NA	NA	NA	NA
8	1645	15122098	NA	NA	NA	NA
18	1647	3319221,	3316087	3324488	IPR011032	GroES-like superfamily
		3319229,			IPR013154	Alcohol dehydrogenase, N-terminal
		3319267			IPR020843	Polyketide synthase, enoylreductase domain
					IPR036292	NAD(P)-binding domain superfamily
4	1648	447128	447024	447052	NA	NA

Table S17: Summary of gene ontology (GO) terms (i.e., classifications) for AIMs which had high *L. melissa* ancestry in the ancient, Jackson Hole hybrids and directional Jackson Hole *Lycaeides* introgression ($\alpha > 0$) in the contemporary Dubois hybrid zone (top 10% in each case). For each gene, the linkage group (LG) (LG 23 = Z), genome scaffold number, positions of SNP or SNPs in or near the gene (SNPs) and start and stop of the gene annotation are given along with the GO term numbers (IDs) and descriptions. Symbols denote whether each term corresponds to a biological process (-b), molecular function (-m), or cellular component (-c).

LG	**Scaffold**	**SNPs**	**Start**	**Stop**	**GO #**	**Description**
11	4	4190884	4190808	4190860	NA	NA
2	11	7335097	7335985	7336153	NA	NA
2	11	8691829	8691651	8691894	NA	NA
2	11	10860558,	10855256	10861383	NA	NA
		10860564			NA	NA
17	309	8389080,	8389311	8389348	NA	NA
		8389096			NA	NA
17	309	12340022	12331408	12343477	GO:0003677-m	DNA binding
	758	2023	NA	NA	NA	NA
21	1095	7019482,	7019198	7025723	GO:0005096-m	GTPase activator activity
		7019493,				
		7019553				
1	1628	2792953	2782773	2797741	GO:0016791-m	Phosphatase activity
1	1628	13677379	NA	NA	NA	NA
23	1631	6116900	6109396	6117970	GO:0007165-b	Signal transduction
10	1639	12335336	12333398	12336053	NA	NA
9	1641	13685914	13678341	13685134	NA	NA
7	1642	11407787	11392125	11409041	GO:0016021-c	Integral component of membrane
8	1645	9366622	9365131	9365852	NA	NA
8	1645	9587985	9587772	9588024	NA	NA
8	1645	15122056	NA	NA	NA	NA
8	1645	15122098	NA	NA	NA	NA
18	1647	3319221,	3316087	3324488	GO:0016491-m	Oxidoreductase activity
		3319229,				
		3319267				
4	1648	447128	447024	447052	NA	NA

Table S18: Table gives a list of sequences used from LepBase version 4 to create the protein homology file for Genome Annotation using MAKER pipeline.

	Sequence name
1	Amyelois_transitella_v1_-_proteins.fa
2	Bicyclus_anynana_nBa.0.1_-_proteins.fa
3	Bicyclus_anynana_v1.2_-_proteins.fa
4	Bombyx_mori_ASM15162v1_-_proteins.fa
5	Calycopis_cecrops_v1.1_-_proteins.fa
6	Chilo_suppressalis_CsuOGS1.0_-_proteins.fa
7	Danaus_plexippus_v3_-_proteins.fa
8	Heliconius_erato_demophoon_v1_-_proteins.fa
9	Heliconius_erato_lativitta_v1_-_proteins.fa
10	Heliconius_melpomene_melpomene_Hmel1_-_proteins.fa
11	Heliconius_melpomene_-_proteins.fa
12	Junonia_coenia_JC_v1.0_-_proteins.fa
13	Lerema_accius_v1.1_-_proteins.fa
14	Limnephilus_lunatus_v1_-_proteins.fa
15	Manduca_sexta_Msex_1.0_-_proteins.fa
16	Operophtera_brumata_v1_-_proteins.fa
17	Papilio_glaucus_v1.1_-_proteins.fa
18	Papilio_machaon_Pap_ma_1.0_-_proteins.fa
19	Papilio_polytes_Ppol_1.0_-_proteins.fa
20	Papilio_polytes_Ppol_1.0_Refseq_-_proteins.fa
21	Papilio_xuthus_Pap_xu_1.0_-_proteins.fa
22	Papilio_xuthus_Pxut_1.0_-_proteins.fa
23	Papilio_xuthus_Pxut_1.0_Refseq_-_proteins.fa
24	Phoebis_sennae_v1.1_-_proteins.fa
25	Plodia_interpunctella_v1_-_proteins.fa
26	Plutella_xylostella_DBM_FJ_v1.1_-_proteins.fa
27	Plutella_xylostella_pacbiov1_-_proteins.fa

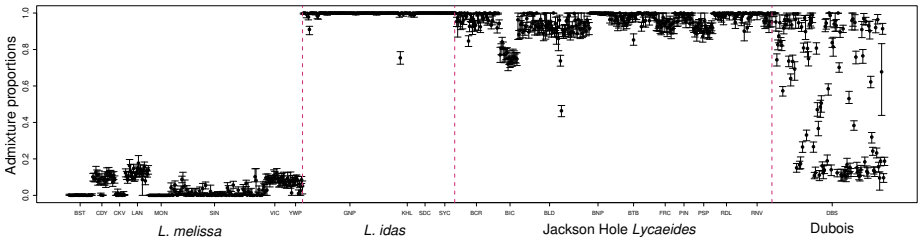

Figure S1: Admixture proportion estimates from `entropy` with $k = 2$ source populations. Points (posterior median) and error bars (95% credible intervals) denote the estimates of the proportion of the genome with *L. idas* ancestry. Tick marks below the plots identify populations based on the population abbreviations in Table S1. Individuals with low coverage are not shown in this figure.

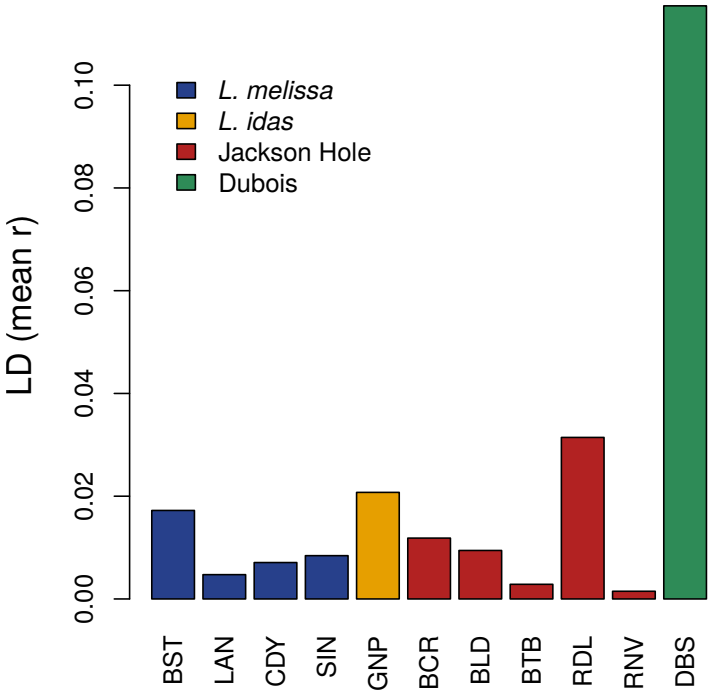

Figure S2: Bars denote the mean value of linkage disequilibrium (LD) for all pairs of ancestry informative SNPs (AIMs) for each population. Here, LD was measured as the Pearson correlation between genotypes at pairs of loci (regardless of physical linkage). LD was calculated based on posterior estimates of genotypes from `entropy`. Genotypes were first polarized such that positive LD indicates a positive association between pairs of alleles that were more common in each of the parental species (i.e., coupling LD). We report r rather than r^2 to retain this sign information. AIMs were defined as those SNPs with an allele frequency difference of at least 0.3 between *L. idas* and *L. melissa*. Only populations with sample sizes of at least 20 were included in this analysis. Population abbreviations are defined in Table S1.

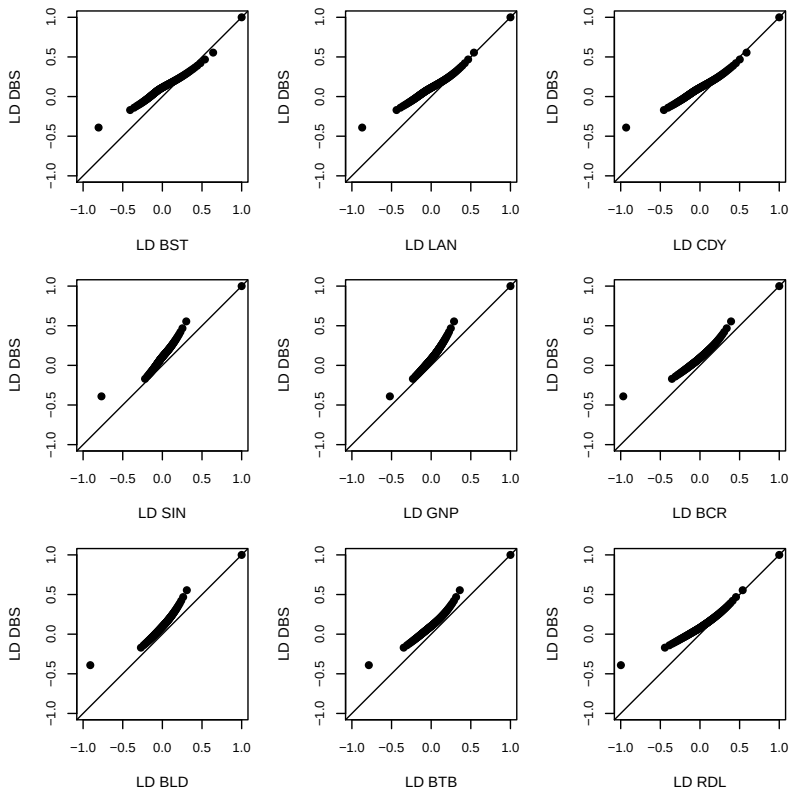

Figure S3: Quantile-quantile plots show the distribution of linkage disequilibrium (LD) for all pairs of ancestry informative SNPs (AIMs) for each population when compared to Dubois. Here, LD was measured as the Pearson correlation between genotypes at pairs of loci (regardless of physical linkage). LD was calculated based on posterior estimates of genotypes from `entropy`. Genotypes were first polarized such that positive LD indicates a positive association between pairs of alleles that were more common in each of the parental species (i.e., coupling LD). We report r rather than r^2 to retain this sign information. AIMs were defined as those SNPs with an allele frequency difference of at least 0.3 between *L. idas* and *L. melissa*. Only populations with sample sizes of at least 20 were included in this analysis. Population abbreviations are defined in Table S1.

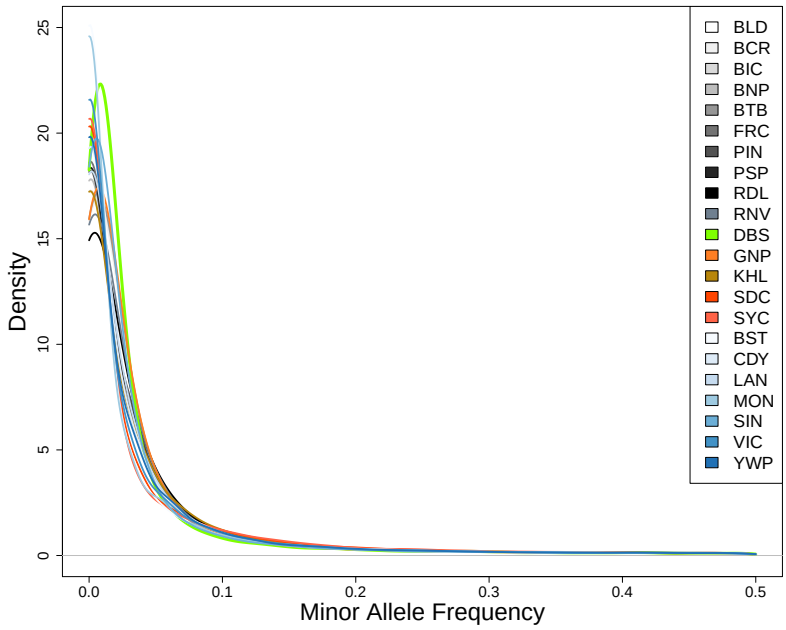

Figure S4: Density plot shows the estimated minor allele frequency distribution for all loci (N = 39,139) for all populations included in this study. The population abbreviations are defined in Table S1.

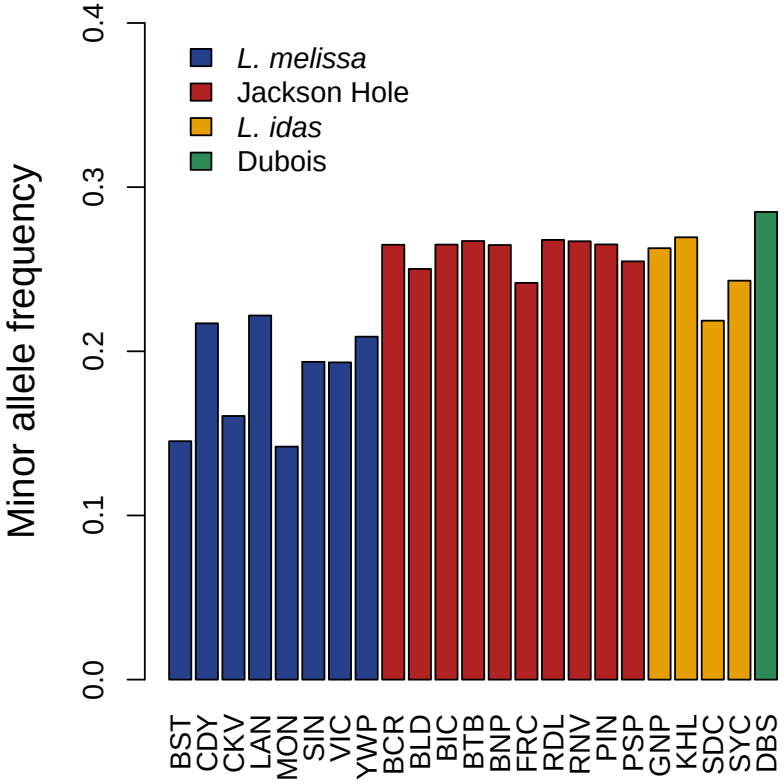

Figure S5: Barplot shows the estimated minor allele frequency distribution for ancestry informative SNPs (AIMs) (N = 1164) for all populations included in this study. The population abbreviations are defined in Table S1.

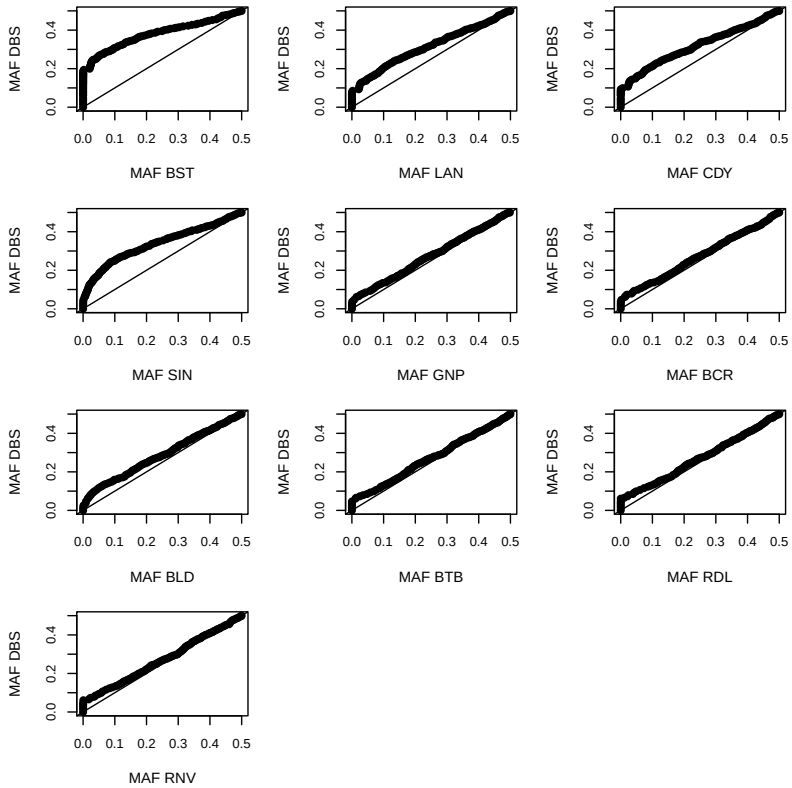

Figure S6: Quantile-quantile plots show the distribution of minor allele frequency (MAF) for all pairs of ancestry informative SNPs (AIMs) for each population when compared to Dubois. Here, AIMs were defined as those SNPs with an allele frequency difference of at least 0.3 between *L. idas* and *L. melissa*. Only populations with sample sizes of at least 20 were included in this analysis. Population abbreviations are defined in Table S1.

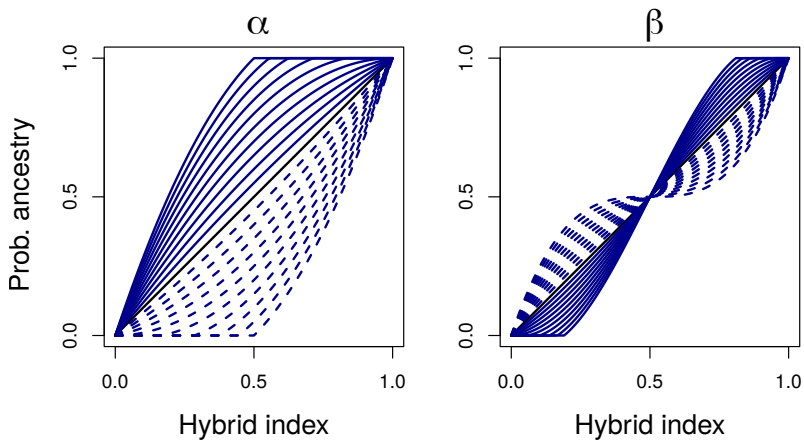

Figure S7: Hypothetical genomic clines. In each panel, the solid black line denotes the probability of ancestry from species one when α and β are zero, which is equal to an individual's hybrid index. In the left panel $\beta = 0$ and α varies from 0.25 to 1.25 (solid blue lines) and from -0.25 to -1.25 (dashed blue lines). Similarly, in the right panel $\alpha = 0$ and β varies from 0.25 to 1.25 (solid blue lines) and from -0.25 to -1.25 (dashed blue lines).

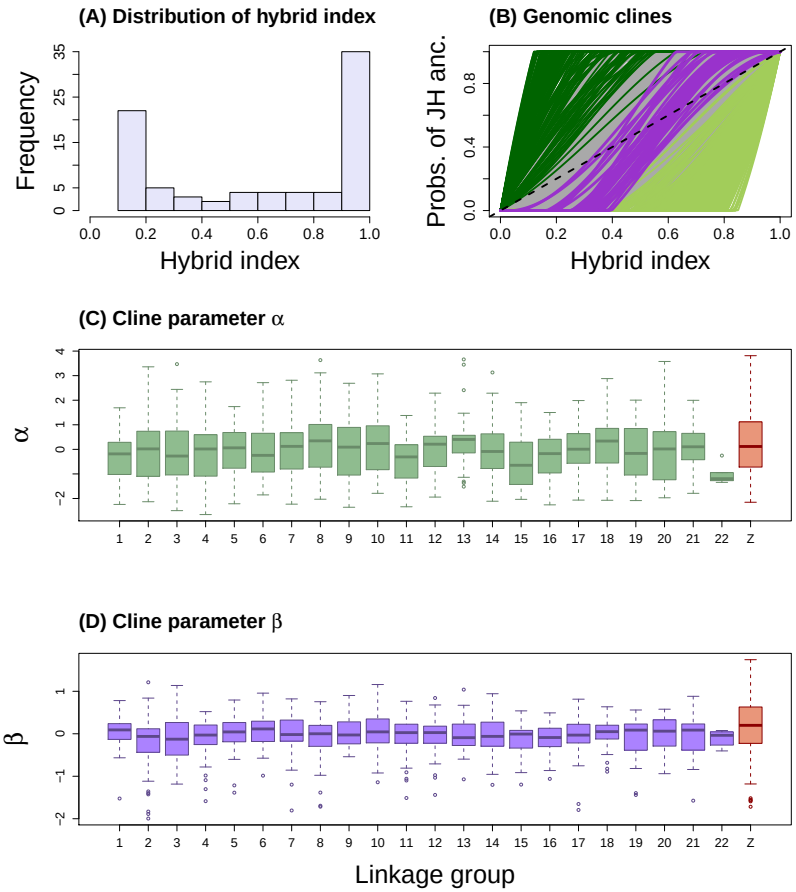

Figure S8: Summary of the genomic clines analysis for **male butterflies**. (A) The histogram depicts the distribution of hybrid indices in the Dubois hybrid zone. (B) This plot shows estimated genomic clines for a subset of ancestry informative SNPs (AIMs). Each solid line gives the estimated probability of Jackson Hole (JH) ancestry for an AIM. Green lines denote cases of credible directional introgression (95% CIs for α that exclude zero) and purple lines denote credible cases of restricted introgression (95% CIs for $\beta > 0$) (gray lines denote clines not credibly different from the genome-average). The dashed line gives the null expectation based on genome-wide admixture. Twenty-seven ($\beta > 0$), 139 ($\alpha > 0$) and 272 ($\alpha < 0$) AIMs showed credible deviations from null expectations, and for $\beta > 0$ and $\alpha > 0$ these were over-represented on the Z chromsome ($\beta > 0$, number on Z = 25, x-fold enrichment = 4.48, $P < 0.001$; $\alpha > 0$, number on Z = 59, x-fold enrichment = 2.06, $P < 0.001$). Boxplots show the distribution of cline paramters α (C) and β (D) across loci for each linkage group.

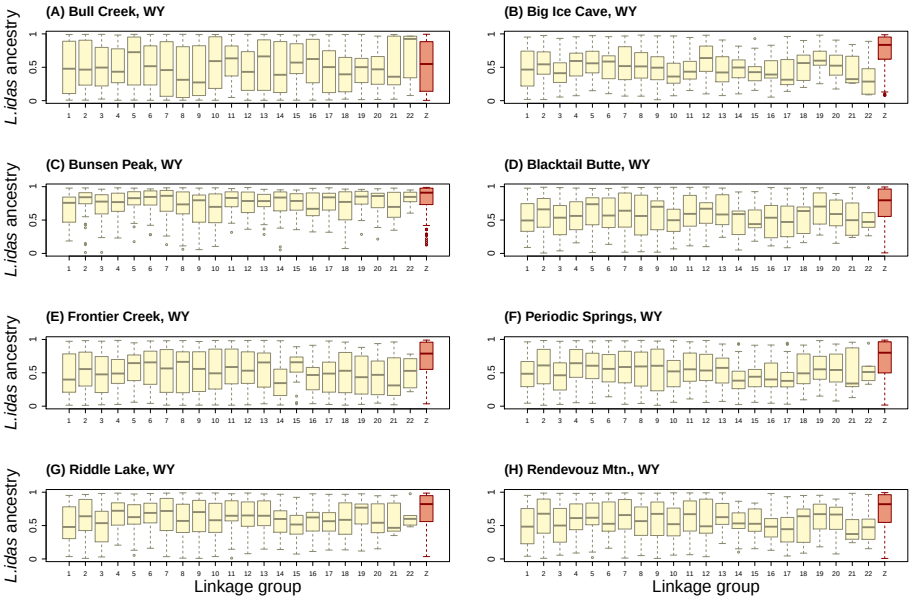

Figure S9: Boxplots show the distribution of *L. idas* ancestry across ancestry informative SNPs (AIMs) for each linkage group in remaining Jackson Hole *Lycaeides* populations. The Z sex chromosome is shown in red.

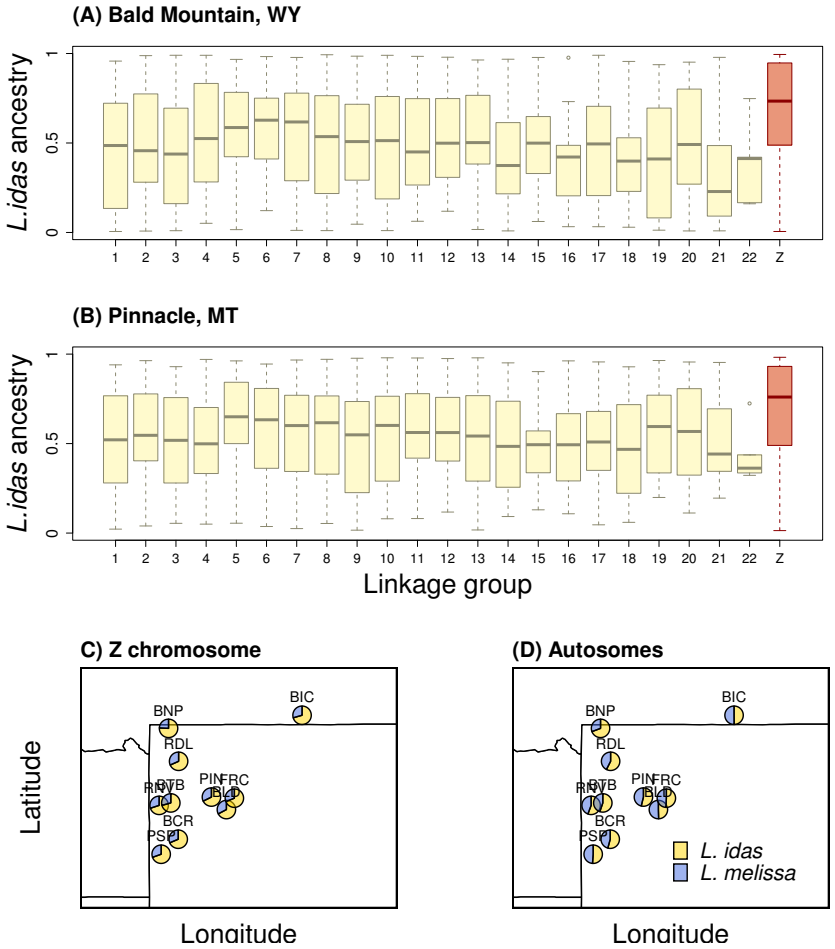

Figure S10: Boxplots show the distribution of *L. idas* ancestry across ancestry informative SNPs (AIMs) for each linkage group in two representative Jackson Hole *Lycaeides* populations–Bald Mountain, WY (BLD) (A) and Pinnacle, WY (PIN) (B). Results are shown for **male butterflies** only. See Fig. S11 for additional populations. The Z sex chromosome is shown in red. Panels (C) and (D) show maps with pie charts reflecting the proportion of *L. idas* and *L. melissa* ancestry (mean) for the Z chromosome (C) and autosomes (D) for each of the ten populations (see Table S1 for population IDs).

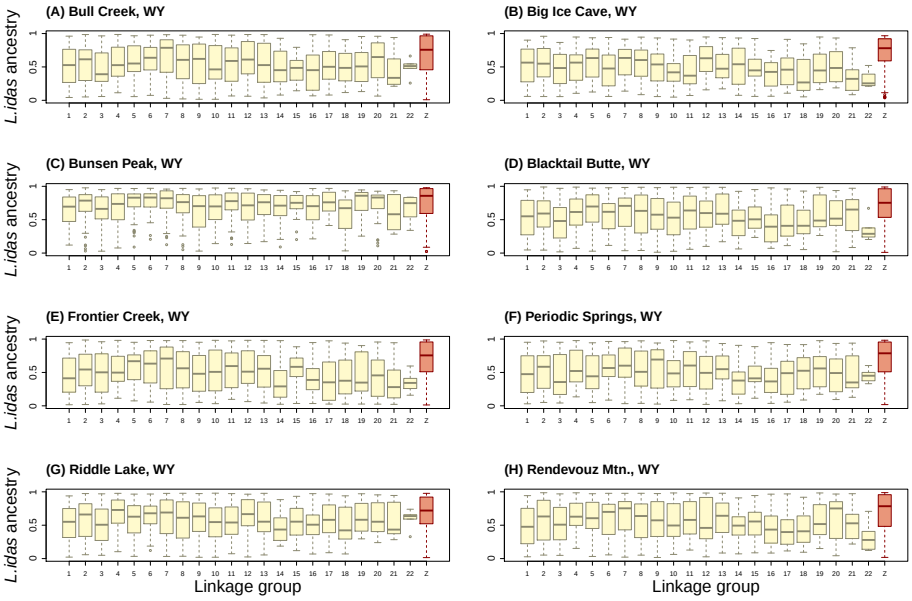

Figure S11: Boxplots show the distribution of *L. idas* ancestry across ancestry informative SNPs (AIMs) for each linkage group in remaining Jackson Hole *Lycaeides* populations. Results are shown for **male butterflies** only. The Z sex chromosome is shown in red.

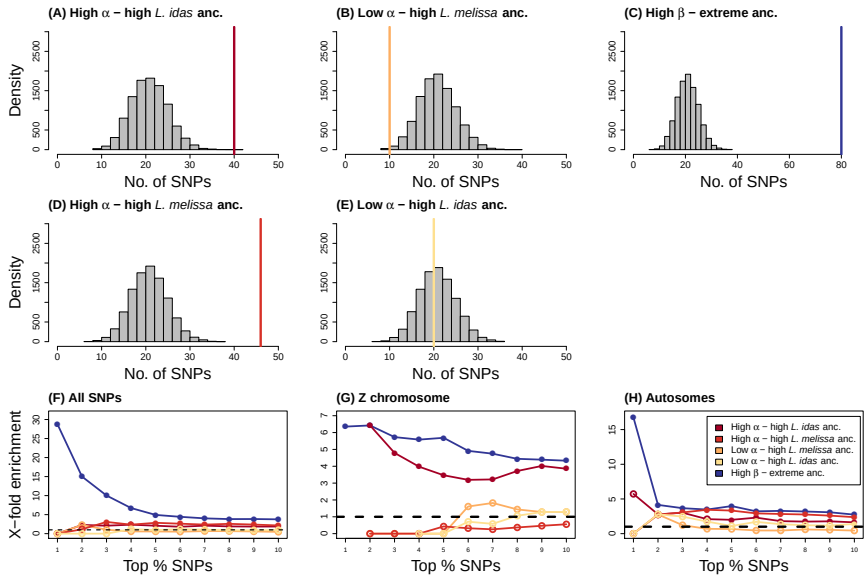

Figure S12: Expected and observed numbers of SNPs with exceptional patterns of introgression in the Dubois hybrid zone and extreme ancestry frequencies in Jackson Hole *Lycaedes*. Here, results are shown for the **2126 SNPs with allele frequency differences of 0.2** or more between *L. idas* and *L. melissa*. Panels A-E show results when considering the top 10% of AIMs in each category. Histograms give null expectations from randomization tests and vertical solid lines show the observed number of AIMs exhibiting a given pattern. Comparisons shown are: directional introgression of Jackson Hole alleles (high α) and high *L. idas* ancestry (in Jackson Hole) (A), directional introgression of *L. melissa* alleles (low α) and high *L. melissa* ancestry (B), restricted introgression (high β) and extremely high *L. idas* or *L. melissa* ancestry (C), directional introgression of Jackson Hole alleles (high α) and high *L. melissa* ancestry (B), and directional introgression of *L. melissa* alleles (low α) and high *L. idas* ancestry (E). Panels F-H show how these results are affected by considering different levels of stringency (i.e., by examining the most extreme 10% to the top 1% of AIMs with each pattern), and when considering only the Z chromosome (G) or only the autosomes (H). Here, circles denote the ratio of the observed to expected overlap from the null, and the circles are filled ($p \leq 0.05$) or not ($p > 0.05$) to denote whether the overlap is greater than expected by chance.

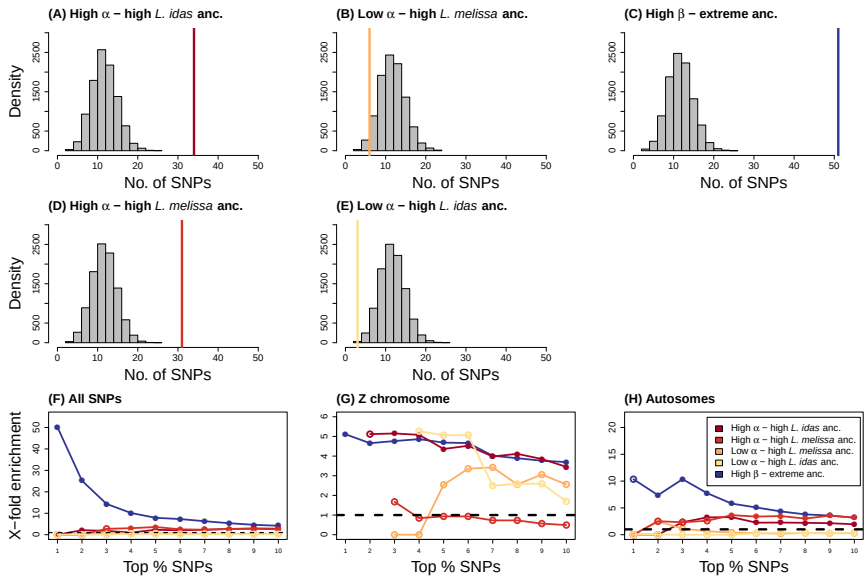

Figure S13: Expected and observed numbers of SNPs with exceptional patterns of introgression in the Dubois hybrid zone and extreme ancestry frequencies in Jackson Hole *Lycaedes*. Here, results are shown for **male butterflies only** and for the **1164 SNPs with allele frequency differences of 0.3** or more between *L. idas* and *L. melissa*. Panels A-E show results when considering the top 10% of AIMs in each category. Histograms give null expectations from randomization tests and vertical solid lines show the observed number of AIMs exhibiting a given pattern. Comparisons shown are: directional introgression of Jackson Hole alleles (high α) and high *L. idas* ancestry (in Jackson Hole) (A), directional introgression of *L. melissa* alleles (low α) and high *L. melissa* ancestry (B), restricted introgression (high β) and extremely high *L. idas* or *L. melissa* ancestry (C), directional introgression of Jackson Hole alleles (high α) and high *L. melissa* ancestry (B), and directional introgression of *L. melissa* alleles (low α) and high *L. idas* ancestry (E). Panels F-H show how these results are affected by considering different levels of stringency (i.e., by examining the most extreme 10% to the top 1% of AIMs with each pattern), and when considering only the Z chromosome (G) or only the autosomes (H). Here, circles denote the ratio of the observed to expected overlap from the null, and the circles are filled ($p \leq 0.05$) or not ($p > 0.05$) to denote whether the overlap is greater than expected by chance.

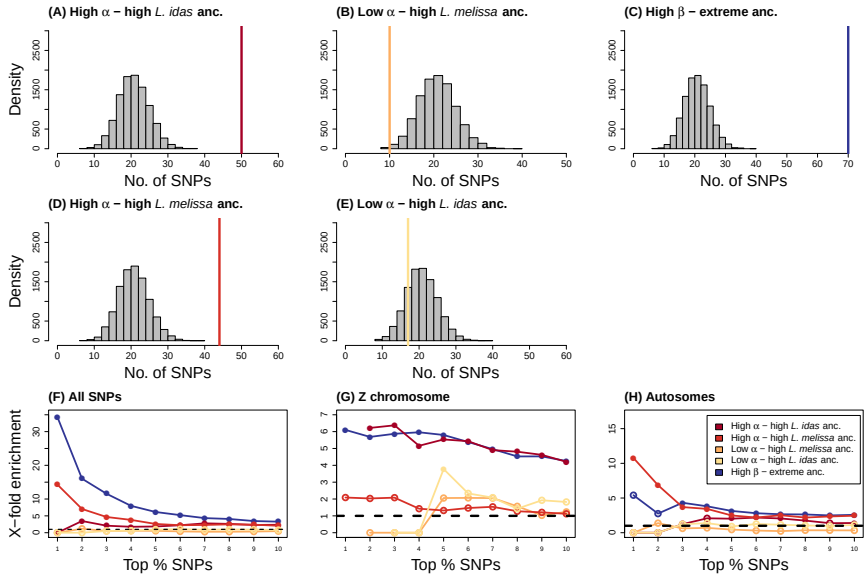

Figure S14: Expected and observed numbers of SNPs with exceptional patterns of introgression in the Dubois hybrid zone and extreme ancestry frequencies in Jackson Hole *Lycaedes*. Here, results are shown for **male butterflies only** and for the **2126 SNPs with allele frequency differences of 0.3** or more between *L. idas* and *L. melissa*. Panels A-E show results when considering the top 10% of AIMs in each category. Histograms give null expectations from randomization tests and vertical solid lines show the observed number of AIMs exhibiting a given pattern. Comparisons shown are: directional introgression of Jackson Hole alleles (high α) and high *L. idas* ancestry (in Jackson Hole) (A), directional introgression of *L. melissa* alleles (low α) and high *L. melissa* ancestry (B), restricted introgression (high β) and extremely high *L. idas* or *L. melissa* ancestry (C), directional introgression of Jackson Hole alleles (high α) and high *L. melissa* ancestry (B), and directional introgression of *L. melissa* alleles (low α) and high *L. idas* ancestry (E). Panels F-H show how these results are affected by considering different levels of stringency (i.e., by examining the most extreme 10% to the top 1% of AIMs with each pattern), and when considering only the Z chromosome (G) or only the autosomes (H). Here, circles denote the ratio of the observed to expected overlap from the null, and the circles are filled ($p \leq 0.05$) or not ($p > 0.05$) to denote whether the overlap is greater than expected by chance.

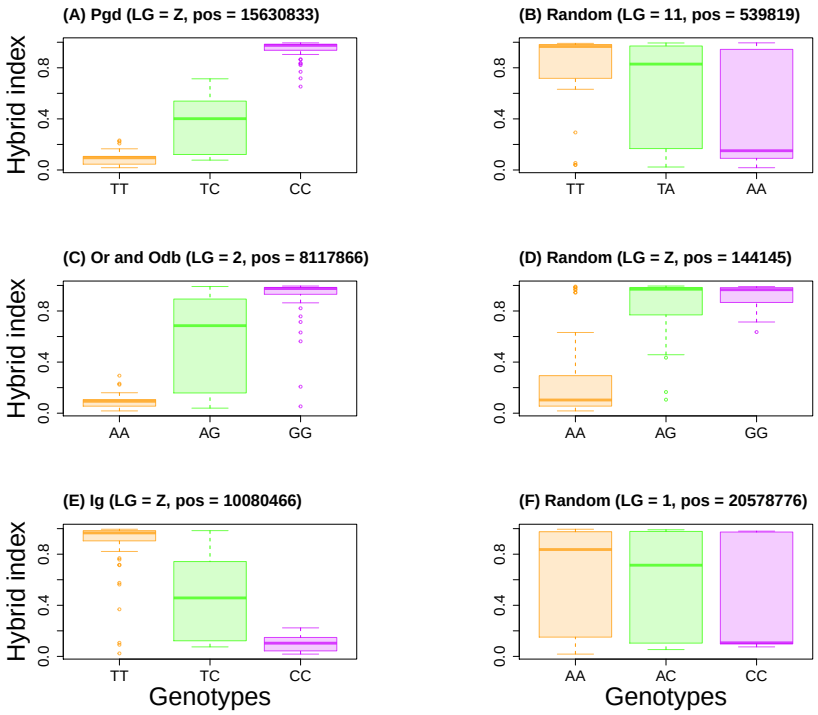

Figure S15: Boxplots shows distribution of hybrid index at each of six AIMs. This includes three of our candidate barrier loci–Pgd = phospogluconate dehydrogenase activity, Or = olfactory receptor activity, Odb = Odorant binding protein, and Ig = Immunoglobulin–and three randomly selected SNPs.

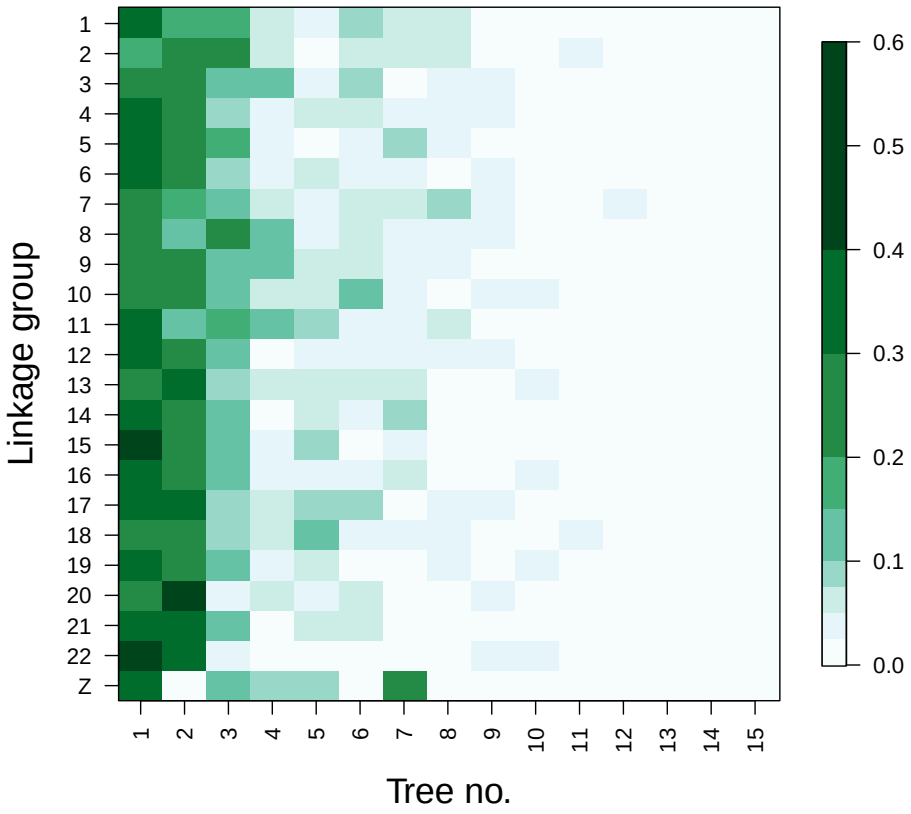

Figure S16: This heatmap shows the proportion of unrooted trees matching each of 15 tree topologies for each linkage group. Unrooted maximum likelihood trees were inferred from non-overlapping 1000 SNP windows designated on each linkage group. Trees 1, 2 and 7 correspond to the topologies shown in Fig. 6 panels (C), (D) and (E), respectively.

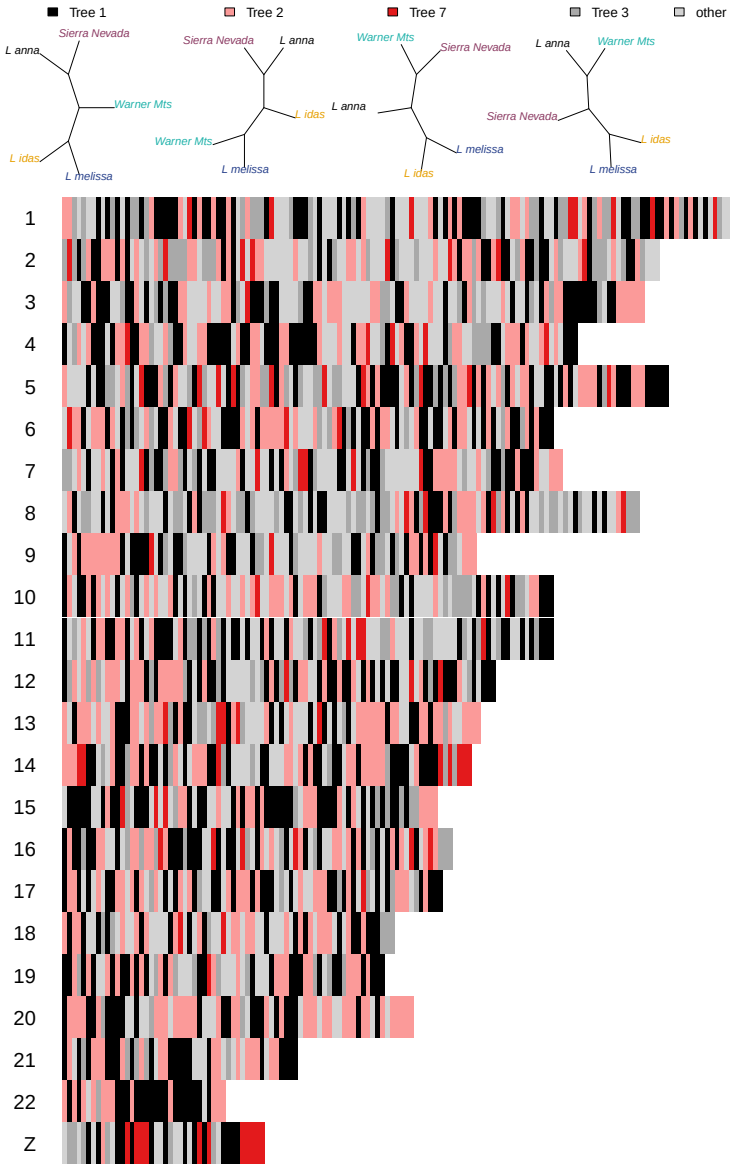

Figure S17: Colored bars denote tree topologies as they vary along linkage groups. Unrooted maximum likelihood trees were inferred from non-overlapping 1000 SNP windows designated on each linkage group. Trees 1, 2 and 7 correspond to the topologies shown in Fig. 6 panels (C), (D) and (E), respectively. Colored bars denote the topology for each 1000 SNP window and these are ordered according physical position along linkage groups. Light gray denotes all topologies except for the trees shown in the legend.