INTRODUCTION

Physical barriers to genetic exchange often arise between interbreeding populations, which may result in reproductive isolation that leads to increased genetic structure between populations; ultimately, such processes may culminate in speciation (Rieseberg & Willis, 2007). However, the instability of biological communities due to changing climates often results in secondary contact of previously isolated lineages. For example, during the Pleistocene, environmental change occurred repeatedly between glacial and interglacial extremes and on a timescale

Abstract

The role of hybridization in diversification is complex and may result in many possible outcomes. Not only can hybridization produce new lineages, but those lineages may contain unique combinations of adaptive genetic variation derived from parental taxa that allow hybrid-origin lineages to occupy unique environmental space relative to one (or both) parent(s). We document such a case of hybridization between two sedge species, Carex nova and Carex nelsonii (Cyperaceae), that occupy partially overlapping environmental space in the southern Rocky Mountains, USA. In the region hypothesized to be the origin of the hybrid lineage, one parental taxon (C. nelsonii) is at the edge of its environmental tolerance. Hybrid-origin individuals display mixed ancestry between the parental taxa—of nearly 7000 unlinked loci sampled, almost 30% showed evidence of excess ancestry from one parental lineage—approximately half displayed a genomic background skewed towards one parent, and half skewed towards the other. To test whether excess ancestry loci may have conferred an adaptive advantage to the hybrid-origin lineage, we conducted genotype–environment association analyses on different combinations of loci—with and without excess ancestry—and with multiple contrasts between the hybrids and parental taxa. Loci with skewed ancestry showed significant environmental associations distinguishing the hybrid lineage from one parent (C. nelsonii), whereas loci with relatively equal representation of parental ancestries showed no such environmental associations. Moreover, the overwhelming majority of candidate adaptive loci with respect to environmental gradients also had excess ancestry from a parental lineage, implying these loci have facilitated the persistence of the hybrid lineage in an environment unsuitable to at least one parent.

KEYWORDS
candidate adaptive loci, genomic cline, genotype–environment association, hybridization, RAD-Seq, redundancy analysis
short enough to prevent reproductive isolation by genetic drift alone (Knowles & Richards, 2005; Kou et al., 2020). When secondary contact occurs, there are a number of potential outcomes for diverging lineages ranging from the breakdown to the strengthening of reproductive barriers (Dion-Côté & Barbash, 2017). The genomic age has confirmed that hybridization between diverging lineages is common (Rieseberg, 1997; Rieseberg & Willis, 2007); an estimated 25% of plant species hybridize with at least one other species (Mallet, 2005). While much of this genomic evidence may indicate transient hybridization events with the potential to impact the evolutionary trajectories of parental species, most events do not result in stable hybrid lineages. However, instances in which hybrids persist illustrate how this process can be a key mechanism in diversification (Seehausen, 2004) and for generating evolutionary novelty (Abbott et al., 2016). Furthermore, studying hybridization may provide valuable insights into the speciation process, including the identification of genomic regions that probably contribute to reproductive isolation (Taylor & Larson, 2019; Wang et al., 2021).

A key insight resulting from genomic analyses of hybridization is that the genomes of hybridizing taxa are not monolithic—regions of the genome may be differentially exchanged between both hybrids and their parental species (Gompert & Buerkle, 2011). Although the role of hybridization in species’ histories varies, genomic exchange facilitated by hybridization may contribute to adaptive evolution (Twyford & Ennos, 2012) and have functional consequences (Abbott et al., 2016). When genetic variation introduced by hybridization events includes adaptive variation, this source of variation may in fact be more effective than new mutation (Abbott et al., 2013; Arnold & Martin, 2009; Grant & Grant, 1994; Kim & Rieseberg, 1999; Kunte et al., 2011; Whitney et al., 2010). The genomic age has advanced our understanding of species boundaries and adaptive introgression, although it remains challenging to link hypothesized adaptive introgression to phenotypic variation and fitness (Suarez-Gonzalez et al., 2018). Nevertheless, powerful insights can be identified in the genomes of extant taxa, which show the effects of hybridization due to histories of ancient hybridization, adaptive introgression and/or hybrid speciation (Taylor & Larson, 2019). For example, genomic data illuminating rapid radiations in African cichlids have revealed that hybridization can provide the genetic novelty that sparks adaptive radiations (Meier et al., 2017). Furthermore, hybridization has enabled fungi to colonize novel environments unsuitable for the parental taxa (Leducq et al., 2016). In some cases, hybrid lineages may readily occupy areas vacated by distributional changes as one or both parents track their niches, often as a response to climatic changes (Kadereit, 2015). In effect, increasingly abundant genomic data confirm the role of hybridization in generating novel gene combinations upon which selection may act (Goulet et al., 2017).

Here, we investigate the consequences of a hybridization event that generated a distinct evolutionary lineage that has spread to occupy a substantial range across the southern Rocky Mountains of western North America. Our focus is on two species in Carex section Racemosae: G. Don, Carex nova L.H. Bailey and Carex nelsonii Mack., and an undescribed, putatively hybrid-origin evolutionary lineage identified during the course of previous research (Massatti & Knowles, 2016). Putative hybrids, while first assumed to represent morphological variation within C. nelsonii, are now hypothesized to represent the southernmost extent of the C. nelsonii range where this parent species is largely absent; as such, these hybrids may occupy environmental space that is different from both putative parental species. Section Racemosae contains at least 10 endemic species across this region, many of which are restricted to montane ecosystems or adjacent, topographically complex landscapes. This diversity probably arose after the start of the Pleistocene (Massatti & Knowles, 2016), during which glacial cycling provided abundant opportunities for complex demographic processes among species’ populations (Hodel et al., 2021; Massatti & Knowles, 2014, 2016). While diversification within section Racemosae is consistent with models of speciation and differentiation in montane systems, the mechanistic basis of diversification remains unknown; understanding such processes may illuminate the origins of diversity in the speciose genus Carex, which occurs in many habitats and regions throughout the world.

To test the hypothesis that a hybridization event between C. nelsonii and C. nova produced an evolutionarily distinct lineage that persists in a unique environment compared to the parents, we first establish the phylogenetic history of the parental taxa and the hybrid lineage. Next, we identify the subsets of the genome (i.e., specific loci) that display either equal ancestry from both of the parental species or excess ancestry from one. In turn, we use the loci displaying excess ancestry to test the hypothesis that hybridization facilitated an adaptive shift that allowed the hybrid lineage to occupy a unique environmental distribution compared to the parental species. Specifically, we use loci with excess ancestry against the background of the parental genomes in a gene-by-environment analytical framework to test for significant relationships between specific loci and a set of independent environmental gradients. If excess ancestry as a result of hybridization allowed the occupation of unique environmental space by hybrids, we predict that unique combinations of putative adaptive loci representative of both parental species will be identified in the hybrid taxon. Our analytical framework clearly supports an independent evolutionary lineage that has spread to occupy a substantial range throughout the southern Rocky Mountains. Moreover, this taxon persists in environments where the parental species that it most closely resembles (morphologically and ecologically; C. nelsonii) does not occur. These patterns suggest that the hybrid taxon may utilize adaptive genetic variation from both parental species that allows it to occupy unique environmental space and supports hybridization as a potential means of diversification in the hyperdiverse genus Carex.

2 | MATERIALS AND METHODS

2.1 | Field sampling and genomic characterization

Samples were field-collected from eight C. nelsonii localities, 15 C. nova localities and five putative hybrid sites (Figure 1). Between two and 10 individuals (mean = 8.5) separated by at least 5 m were
collected at each sampling locality (Tables S1 and S2). Putative hybrid-origin individuals collected and keyed out to *C. nelsonii* were only identified as hybrid-origin during the course of population genetic analyses. A total of 237 individuals—54 *C. nelsonii*, 136 *C. nova* and 47 putative hybrids—were used for genomic analyses (Figure 1; Tables S1 and S2).

We performed double-digest RAD-Seq (Peterson et al., 2012) to obtain anonymous genomic loci. We used two restriction enzymes, *Eco*RI and *Mse*I, to digest genomic DNA, and ligated Illumina adaptor sequences and unique 10-bp barcodes to restriction sites. The ligation products for each library were pooled and PCR (polymerase chain reaction)-amplified for 12 cycles, and 400–500-bp fragments were size selected using a Pippin Prep (Sage Science). All libraries were sequenced on single-end Illumina HiSeq 2500 runs at The Centre for Applied Genomics (Hospital for Sick Children, Toronto, Ontario). Raw sequencing reads were processed using *ipyrad* version 9.42 (Eaton, 2014; Eaton & Overcast, 2020) in a single assembly that combined both species and the putative hybrids (Table S2). The combined assembly approach facilitated the tracking of allele frequencies across all individuals and populations in downstream analyses. The *ipyrad* assembly was filtered using *vcftools* (Danacek et al., 2011) to allow 40% missing data per site (−max-missing = 0.6) and require a minor allele count of ≥2 (−mac = 2). The resulting data matrix consisted of 6946 unlinked single nucleotide polymorphisms (SNPs) (i.e., one randomly selected SNP per RAD-Seq locus). Because we were interested in the effects of putative adaptive loci associated with hybridization and not loci associated with selection within one of the parental species, we used *pcadapt* (Luu et al., 2017) to remove *F*~ST~ outliers identified within either *C. nelsonii* or *C. nova*; in total, we removed 206 loci, which left 6740 unlinked SNPs that were used in most subsequent analyses, except where noted. To verify that removing *F*~ST~ outliers did not bias our downstream results, we ran several analyses both with and without *F*~ST~ outliers, including analyses of excess ancestry, all genetic diversity and differentiation statistics, and redundancy analyses, as noted below.

A separate *ipyrad* assembly was performed to add an outgroup (*Carex chalciolepis*) suitable for rooting the phylogeny of *C. nelsonii* + *C. nova* + hybrids and for use in downstream analyses (see hybridization analyses below). This assembly included the 237 individuals referenced above plus 152 individuals of *C. chalciolepis* and utilized the same filtering strategy as the first assembly. The resulting SNP data matrix consisted of 6069 unlinked SNPs, which were used in the *svdquartets* and *treemix* analyses. The authors of
HYDE recommend including invariant sites in the genetic data set, so we used all 69,373 sites from the assembly with the outgroup in the HYDE analysis (see below).

2.2 | Environmental characterization

We characterized the environmental niche of each species—C. nelsonii, C. nova and putative hybrids—by performing a principal components analysis (PCA) on environmental variables across the study region. The environmental PCA was run using the “dudi.pca” function with centring and scaling in the R package ade4 (Dray & Dufour, 2007). Specifically, we included 17 of 19 “bioclim” layers (Hijmans et al., 2005; http://worldclim.org) with a spatial resolution of 30-arc seconds; BIO8 and BIO9 were excluded because of bimodal distributions in the study region. The R packages dismo (Hijmans, 2017) and raster (Hijmans, 2019) were used to extract environmental data from GPS points representing individuals at each of the sampling locations (Table S3). The first three principal components, which explain 89.9% of the variation, were used to define the environmental space occupied by each taxon.

2.3 | Genetic relationships between individuals and populations

The “populations” program in STACKS version 2.41 (Catchen et al., 2013) was used to estimate per-locus $F_{ST}$, pairwise $F_{ST}$, nucleotide diversity ($\pi$), observed heterozygosity ($H_0$) and % polymorphic loci. Herein, we used these statistics to compare taxa and sets of loci, but we caution against comparing their absolute magnitudes to values in other studies because they are sensitive to filtering parameters and the markers used. We estimated pairwise $F_{ST}$ between all sampling localities within taxa as well as between C. nelsonii, C. nova and putative hybrids. After discovering differences in occupied environmental space and in summary statistics between hybrids from different populations and/or geographical regions (Figure 1), we reran analyses considering subsets of hybrid groups. The above population genetic statistics were also calculated separately for sets of loci defined by excess ancestry due to hybridization, or a lack thereof (see “Identifying loci with excess ancestry” below).

Furthermore, we implemented the Bayesian clustering program STRUCTURE (Pritchard et al., 2000) to infer population assignment and identify signals of hybridization. Specifically, we used an admixture model with correlated allele frequencies for $K = 1$–6 and ran 1000,000 Markov chain Monte Carlo (MCMC) generations with a burnin of 200,000 generations. We also investigated genetic similarity among individuals using a PCA implemented in the R packages SNPRelate (Zheng et al., 2012) and vegan (Oksanen et al., 2017). SVDQUARTETS (Chifman & Kubatko, 2014) was used to infer phylogenetic relationships between all C. nelsonii, C. nova and hybrid individuals, as well as a rooted phylogeny using C. nelsonii, C. nova, hybrids and an outgroup, C. chalciolepis. For consistency with previous analyses, STRUCTURE, PCAs and unrooted SVDQUARTETS analyses used the SNP data set with the 206 loci considered $F_{ST}$ outliers in one parent removed.

Hybridization among taxa was assessed using HYDE (Hybrid Detector; Blischak et al., 2018) and TREEmix (Pickrell & Pritchard, 2012). HYDE uses phylogenetic invariants arising under a coalescent model that includes hybridization to detect and assign probability of hybridization of the three ingroup taxa (i.e., C. nelsonii, C. nova and the putative hybrids). The parameter $\gamma$ is the probability of gene trees having a hybrid population sister to parent A arising under the parental population trees while $1 - \gamma$ is the probability that the hybrid population is sister to parent B. We ran HYDE analyses exhaustively on all possible combinations of taxa, with C. chalciolepis set as the outgroup. To further investigate signals of hybridization, HYDE analyses were complemented with TREEmix, an approach that incorporates reticulation in a phylogeny. TREEmix uses allele frequencies and a Gaussian approximation of genetic drift to maximize the likelihood of a population covariance matrix while allowing both population splits and admixture via a migration parameter. The change in the likelihood score as the number of migration events ($m$) ranged from zero to five was assessed in line with ipyrad analysis tools (https://ipyrad.readthedocs.io/en/latest/API-analysis/cookbook-treemix.html; Eaton & Overcast, 2020) and the optimal $m$-value was inferred using OPTM (Fitak, 2021). We investigated a range of cutoffs (50–50%) for the minimum percentage of samples required to be present in each species and used 10 random starting seeds for each run for a particular $m$-value. If one or more migration events are inferred, a reticulate topology explains the data better than a purely bifurcating one and is therefore consistent with a hybridization history. Based on STRUCTURE and SVDQUARTETS analyses, we used several strategies for grouping hybrid populations in the TREEmix analysis.

2.4 | Identifying loci with excess ancestry

A genomic cline approach, implemented in the software BGC (Gompert & Buerkle, 2012), was used to identify loci with alleles displaying excessive amounts of ancestry from one parent relative to the average genomic background. The BGC analysis defined parental populations as those with a $K = 3$ STRUCTURE-Q-score $>0.9$, as recommended by the developers, resulting in the exclusion of three C. nova populations (Lizard Head, Pike’s Peak, Red Lakes; see Figure 2). In our use of this model, a positive genomic cline parameter $\alpha$ describes an increase in the probability that a hybrid has an excess of C. nova ancestry relative to the expectation based on the hybrid index ($h$), whereas a negative $\alpha$ represents a deficit of C. nova ancestry. The genomic cline parameter $\beta$ specifies an increase (positive $\beta$) or decrease (negative $\beta$) in the rate of transition from low to high probability of C. nova ancestry as a function of hybrid index ($h$). After convergence among 10 independent MCMC runs, each having 25,000 burnin generations
followed by 50,000 generations, we assessed the $\alpha$ and $\beta$ parameter of each locus. We identified loci displaying significant departures from null expectations (i.e., equal ancestry of alleles from each parent) by assessing whether 95% confidence intervals around estimates of $\alpha$ or $\beta$ overlapped with zero. Such loci with excess ancestry from either parent as defined by exceptional values of $\alpha$ are termed “outlier loci.” For brevity, outlier loci with excess $C. nova$ ancestry are referred to as “$C. nova$-outliers” and outlier loci with excess $C. nelsonii$ ancestry are referred to as “$C. nelsonii$-outliers.” Loci with no detectable excess ancestry are hereafter referred to as “inlier loci.”

2.5 | Environmental associations of loci

We used redundancy analysis (RDA), a multivariate ordination method that can simultaneously compare multiple loci and environmental variables, to determine the effect of environmental gradients on genetic variation in different sets of loci (Forester et al., 2018). RDA is capable of identifying signatures of weak and/or multi-locus selection and adaptation (Forester et al., 2018; Rellstab et al., 2015), and this approach has a documented low rate of false positives and high rates of true positives across a range of demographic histories (Capblancq et al., 2018). Here, we used RDA to test if loci displaying excess ancestry from one parent disproportionately influence the climatic niche of the hybrid taxon. In our use of RDA, genetic and environmental data are analysed using multivariate linear regression resulting in a matrix of fitted values (Legendre & Legendre, 2012). Next, input predictors (i.e., environmental variables) are constrained to test if individual input axes explain more of the variation in a response variable (in this case genotype) compared to a PCA of the input axes (Forester et al., 2018; Massatti & Knowles, 2020; Vanhove et al., 2021). To avoid pseudoreplication, we used genetic data based on populations instead of individuals; population-level allele frequencies were calculated using the “make-freq” function in the R package adegenet (Jombart & Ahmed, 2011). The environmental input variables used were the PC axes from an environmental PCA constructed in a similar fashion as described above in “Environmental characterization,” except this time a single GPS coordinate was used per population to ensure one-to-one correspondence between genetic data (i.e., population-level allele frequencies) and environmental data in the RDA.
If hybridization allows the expansion of the occupied environmental space by the hybrid compared to one or both parents, we expect significant RDA results when investigating outlier loci between the hybrid and a parent. At the same time, we would not expect a significant environmental association when using inlier loci from the hybrid plus a parent. Accordingly, genotype–environment associations were tested using RDA for each set of outlier loci (i.e., C. nelsonii outliers, C. nova-outliers) first considering the hybrid plus each parent. To ensure that any detected significant results using outlier loci in the hybrids are meaningful, we also used RDA to analyze all other possible combinations of loci sets (i.e., inlier loci, all loci) and taxa (i.e., the hybrid separately, each parent separately and between the parents) as controls. Each overall RDA model and the individual RDA axes were tested for significance using permutation tests. Significant RDA axes are considered evidence that the environmental input variables explain a substantial amount of genetic variation.

For any RDAs that were significant, we identified putative adaptive loci across environmental gradients by following the methods of Forester et al. (2018). Candidate adaptive loci may load significantly on any of the three environmental PC axes, and we assessed the loadings of candidate adaptive loci to infer environmental conditions that may be associated with adaptation. We used the RDAs including inlier loci as the background against which to test for candidate adaptive loci due to hybridization. To test the hypothesis that excess ancestry loci within the hybrid taxon allow it to occupy unique environmental space compared to the parental taxon that it most closely resembles (C. nelsonii), we visualized sampling sites of C. nelsonii and the hybrid taxon in RDA space using only C. nelsonii outliers and C. nova-outliers. Candidate adaptive loci were defined as those outside of a two standard deviation cutoff relative to the background of inlier loci as determined by the RDA of C. nelsonii + the hybrid using only inlier loci. To clarify the distinction between outlier loci with respect to ancestry (i.e., a outliers detected using BGC) and loci that are statistical outliers in RDAs, we hereafter refer to RDA-outliers as "candidate adaptive loci."

3 | RESULTS

3.1 | Genetic characterization of lineages and hybrid diagnosis

The HYDE analyses confirmed the hypothesized hybrid origin of the five putative hybrid populations and rejected hybrid status for all populations identified as C. nova and C. nelsonii (Table 1). Hybrid indices were skewed towards C. nova ancestry (average hybrid index = 0.6, where 0 = pure C. nelsonii and 1 = pure C. nova; Table S4). STRUCTURE results using a K = 2 model support the hypothesis that the total genetic diversity across taxa represents the gene pools of two species; hybrid individuals largely clustered with C. nelsonii (Figure 2). The K = 3 STRUCTURE solution clearly separates the five hybrid populations, with hybrid Q-scores corroborating the HYDE results (Figure 2). In the genetic PCA, variation on PC1 corresponds to the K = 2 STRUCTURE plot, with C. nova distinguished from C. nelsonii + hybrids (Figure S1). Notably, individuals from the Mt Oso, Red Lakes and Lizard Head hybrid populations are more diffuse in PC space than individuals from the Medicine Bows and Flat Tops hybrid populations (Figure S1). PC2 differentiates between the hybrid and C. nova + C. nelsonii, whereas PC3 shows the hybrid individuals intermediate between C. nova and C. nelsonii, and in some cases overlapping with the genetic space occupied by C. nova (Figure S1).

The SVUQARTETS species tree recovered a strongly supported clade (bootstrap score = 100%) containing all C. nelsonii individuals as sister to a strongly supported clade containing all hybrids from Medicine Bows and Flat Tops, and most individuals from Lizard Head and Red Lakes and one Mt Oso individual (Figure 3). All hybrid individuals from Medicine Bows and Flat Tops were in a strongly supported monophyletic group (bootstrap score = 94%) nested within the hybrid clade (Figure 3); hereafter this lineage is referred to as the "northern hybrids" to promote discussion of evolutionary hypotheses. Nine out of 10 Lizard Head hybrid individuals plus eight out of 10 Red Lakes hybrids formed a clade with high bootstrap support (93%) sister to the northern hybrids; for brevity, this lineage is hereafter referred to as "core southern hybrids" (Figure 3). Southern hybrid individuals outside of the core southern hybrid clade—including all Mt Oso hybrids, as well as one Lizard Head individual and two Red Lakes individuals—are described as "Mt Oso-like hybrids" hereafter and may fall outside the hybrid clade due to more recent backcrossing events with C. nova (Figure 2); regardless, these patterns indicate a single origin for the hybrid-origin taxon.

The clade consisting of C. nelsonii plus most hybrids is sister to a clade containing all C. nova plus two hybrid individuals from Mt Oso (Figure 3). These results, including low support at nodes and the various placement of hybrid individuals, are readily explained by the various patterns of ancestry coefficients in some hybrid and southern C. nova individuals displayed in Figure 2. Phylogenetic relationships inferred using the C. chalciolepis-rooted data set support the SVUQARTETS inference (Figure S2), although there are some minor differences, which is common at such shallow-scale divergences (Wang et al., 2017). The TREEMIX phylogeny inferred the same branching topology as the SVUQARTETS tree (Figure 3; Figures S2 and S3) and consistently inferred C. nelsonii as sister to the northern and all southern hybrids with one migration event from C. nova to the southern hybrids (Figure 3). Excluding the Mt

| TABLE 1 | Results from hybrid detector (HYDE) for the three Carex lineages to test hybrid status using phylogenetic invariants arising under a coalescent model with hybridization |
|----------------|----------------|----------------|----------------|----------------|
| P1 HYDE P2     | Z-score | p-value | γ              |
| C. nelsonii Hybrid C. nova         | 3.119  | .001   | 0.822          |
| C. nelsonii C. nova Hybrid         | −9.725 | 1.000  | 0.562          |
| Hybrid C. nelsonii C. nova         | −2.559 | .995   | 1.376          |

Note: The test statistics show the results of testing the null hypothesis that $γ = 0$, where the parameter $γ$ describes the probability of hybrid ancestry from one parent while $1 − γ$ represents the probability of hybrid ancestry from the other parent. Only the case in which the hybrids were tested as the hybrid taxon rejected the null hypothesis.

Figures S2 and S3
Oso-like hybrids or treating them as a distinct entity did not have an impact on the branching topology (Figure S3). When the northern, core southern and Mt Oso-like hybrids were considered as separate groups, the Mt Oso-like hybrids were sister to Carex nova, but Carex nelsonii remained sister to the northern plus core southern hybrids, with one migration event from Carex nova to the core southern hybrids (Figure S3).

Nucleotide diversity ($\pi$) across all 6740 loci ranged from 0.021 (Carex nova–Uintahs) to 0.186 (hybrid–Mt Oso). At the taxon level, genetic diversity of Carex nova ($\pi = 0.102$) is nearly twice that of Carex nelsonii ($\pi = 0.055$), while the highest diversity is represented by all hybrids ($\pi = 0.161$) (Table S5). The number of polymorphic sites in Carex nova (4025) is nearly four times greater than in Carex nelsonii (1119), while the hybrids have an intermediate value (3507) closer to that...
of C. nova than C. nelsonii. $F_{ST}$ was lowest between the C. nova and hybrid populations (0.167), higher between C. nova and C. nelsonii (0.270), and highest between C. nelsonii and all hybrids (0.311) (Table S6). Genetic diversity and differentiation statistics for each set of loci, and using different sets of hybrid individuals, are shown in Tables S5 and S6.

3.2 | Environmental characterization

The environmental space occupied by C. nelsonii is substantially smaller than that of C. nova (Figure 4; Figure S4). Certain bioclimatic variables are strongly (i.e., $r^2 > .45$) and significantly ($p < .001$) associated with the PC axes (Table S7; Figure 4). Despite substantial overlap on PC1, there is a tendency for C. nelsonii to occupy more positive values on this axis, which is associated with lower temperatures and greater precipitation, while C. nova extended further into negative PC1 values indicative of higher temperatures and less precipitation (Figure 4). C. nelsonii's distribution across PC2 is largely contained within the variation represented by C. nova, which also occupies a substantial and unique distribution across negative values of PC2 (corresponding to habitats with higher diurnal temperature range and higher precipitation seasonality). The hybrid lineage occupies a similarly sized, but largely distinct, environmental space compared to C. nelsonii (Figure 4), overlapping with both parental species in a small region with intermediate values of both PC1 and PC2. This region corresponds to the far northern portion of C. nova's range in northern Colorado and southern Wyoming (Figure 1). In other words, the northern hybrids, which are the most differentiated from both parental species (Table S6), overlap with the environmental space of each parental taxon; the Lizard Head, Red Lakes and Mt Oso hybrids from the southern part of the study range overlap only with C. nova in environmental space not occupied by C. nelsonii (Figure 4).

3.3 | Loci with signatures of excess ancestry from a parental lineage

Genomic cline analyses identified a substantial number of loci (29.6%) with significantly positive and negative $\alpha$-values indicating excess ancestry from either C. nova or C. nelsonii, respectively; no extreme $\beta$-values were detected. Out of 6740 loci, 1027 had excess C. nova ancestry and 971 had excess ancestry C. nelsonii, leaving 4742 loci showing equal ancestry from both parents ("inlier loci"). Outlier loci with excess C. nova ancestry are hereafter referred to as "C. nova-outliers" and outlier loci with excess C. nelsonii ancestry are referred to as "C. nelsonii-outliers," while loci with no detectable excess ancestry are hereafter referred to as "inlier loci." Using all loci, genetic differentiation between the parental species, as measured by pairwise $F_{ST}$, is positively correlated with the magnitude of both $\alpha$ ($r^2 = .030, p < 2.2e-16$) and $\beta$ ($r^2 = .080, p < 2.2e-16$) estimates on a per-locus basis. These regression coefficients are small but similar to results from previous studies (Parchman et al., 2013). When the 206 loci identified as $F_{ST}$ outliers in either parent were included, the distribution of loci was qualitatively similar: out of 6946 loci, 1061 were C. nova-outliers, 998 were C. nelsonii-outliers and 4887 were classified as inlier loci.

3.4 | Associations of loci with environmental conditions

The allelic makeup of the hybrid-lineage sampling sites vs. C. nelsonii sampling sites clearly distinguishes populations of C. nelsonii from hybrids (Figure 5a), with both sets of outlier loci showing significant associations with environmental conditions. The overall RDA model and the RDA1 canonical axis were significant for both (Table 2). Simultaneously, no environmental associations of inlier loci distinguished C. nelsonii from the hybrid lineage. Hybrid genotypes at outlier loci are generally associated with higher temperatures and precipitation than loci in C. nelsonii; the hybrid lineages trend towards positive PC1 (positively correlated with many precipitation variables: BIO12, BIO13, BIO16, BIO17, BIO18, BIO19; hereafter referred to as "precipitation axis") and PC2 (positively correlated only with temperature variables: BIO5, BIO10; hereafter referred to as "temperature axis") space (Figure 5a). There was also an association between the alleles of the two parental species and environmental gradients when using all 6740 loci, and either outlier set of loci. An RDA using inlier loci was not significant, so the significance of the RDA using all loci may have been driven by outlier loci. However, when $F_{ST}$ outliers within either parent were included (i.e., the 6946 loci data set), the RDA comparing both parents using inlier loci was significant (Table S8), which may be attributed to the influence of loci that were $F_{ST}$ outliers with respect to differentiation. No other comparisons were significant for any other combinations of taxa, and all RDAs using inlier loci were non-significant (Table 2).

We investigated SNPs from the significant RDAs using outlier loci with hybrids and C. nelsonii in more detail, revealing that candidate adaptive loci were overwhelmingly represented by the two sets of outlier loci as opposed to inlier loci (Figure 5b). Against the background of inlier loci, we applied a two standard deviation cutoff, which is appropriate to identify loci experiencing different strengths of selection (one of the core strengths of RDA); 356 C. nelsonii-outlier loci and 251 C. nova-outlier loci were identified as candidate adaptive loci (607 out of 1998 outlier loci; Figure 5b, Table 3). In contrast, only 10 out of 4742 inliers surpassed the two standard deviation cutoff. As such, >98% (607 out of 617) of candidate adaptive loci belong to the two sets of outlier, as opposed to inlier, loci.

Candidate adaptive loci loaded on both the temperature and precipitation axes (Figure 5b, Table 3). Because results from environmental PCAs using multiple GPS coordinates per sampling location informed the description of environmental space (Figure 4; Figure S4) whereas the identification of candidate adaptive loci in RDAs used one GPS point per sampling location (Figure 5), we consider how candidate adaptive loci identified by RDAs correspond to the overall
environmental space of the three taxa. Candidate adaptive loci loading positively on the temperature axis (PC2 in the RDA; Figure 5) correspond to the lower left quadrant of the environmental PCA (Figure 4), while candidate adaptive loci loading negatively on the temperature axis correspond to the upper right quadrant of the environmental PCA. Meanwhile, candidate adaptive loci loading positively on the precipitation axis (PC1 in the RDA; Figure 5) correspond to the lower right quadrant of the environmental PCA, while candidate adaptive loci loading negatively on the precipitation axis correspond to the upper left quadrant of the environmental PCA. Crucially, all candidate adaptive loci that load on the temperature or precipitation RDA axes—except those negatively correlated with the temperature axis—align with space in the environmental PCA (Figure 4) that does not correspond to an environment occupied by C. nelsonii; in total, 499 of 607 candidate adaptive loci correspond to environmental space unsuitable for C. nelsonii (Figures 4 and 5b, Table 3).
DISCUSSION

Our detailed examination of anonymous genomic loci within a hybrid and its two parents not only solidifies the role of hybridization in the formation of a new lineage of montane sedges, but it also shows that hybridization has facilitated an adaptive shift allowing hybrid individuals to occupy unique environmental conditions compared to the parent that it most closely resembles in terms of morphology and habitat preference. As such, our work directly addresses the role of hybridization in promoting diversification. Our results suggest that rather than simply representing a breakdown of reproductive isolation (and the potential for the loss of diversity through the merging of divergent species’ lineages), the hybridization studied herein represents a case in which alleles contributing to adaptation have been uniquely combined to promote the occupation of a new climatic niche. Specifically, the hybrids are recognized genomically as an evolutionary lineage distinguishable from the parental species *Carex nelsonii* and *C. nova*. Moreover, outlier loci with respect to ancestry show significant environmental associations distinguishing the hybrid lineage from *C. nelsonii*, whereas other loci do not. This suggests that hybridization has played a role in adaptive divergence. By mapping the environmental associations to axes of environmental variation, we also show the potential role of candidate adaptive loci in facilitating a potentially adaptive environmental shift as well as the nature of this environmental shift (e.g., contributing to a shift along a precipitation vs. temperature gradient) (Figures 4 and 5, Table 3; Table S7). Below, we discuss how this evidence contributes to our understanding of hybridization as a mechanism for diversification in montane sedges, and more generally how the dissection of the history of hybridization, and specifically the heterogeneous contribution of parental genomes to hybrid taxa, can be quantified analytically to identify candidate adaptive loci that have spread between species.

4.1 Candidate adaptive loci acquired through hybridization

The genomes of many species show evidence of ancient and/or recent hybridization (Taylor & Larson, 2019). However, such hybridization is not always adaptive and could simply reflect the permeability of species boundaries (Gompert et al., 2017; Twyford & Ennos, 2012). As such, additional evidence is required to evaluate whether there is an

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**Figure 5** (a) RDA plots for *Carex nelsonii* and the hybrid lineage using the two sets of loci identified as outlier loci with excess ancestry from one of the parent species; *Carex nova*-outliers (1027 loci) are shown in the top plot, and *C. nelsonii*-outliers (971 loci) in the bottom plot. Each plot shows RDA axis 1 vs. RDA axis 2, with SNPs depicted by small grey dots. Populations are shown by colour-coded dots. In both plots, the overall RDA and axis 1 were significant. The positions of the populations on RDA1, but not RDA2, indicate significant differences in the environmental association of the alleles in a population. (b) Detailed relationships among SNPs from the RDA plots (a). Inlier loci with no excess ancestry from either parent are shown as white dots, while candidate adaptive loci that showed a significant association with either the precipitation axis (PC1) or the temperature axis (PC2) are colour-coded dots. Maroon- and periwinkle-coloured dots mark SNPs correlated with PC1, and red and navy dots mark SNPs correlated with PC2. Candidate adaptive loci with an excess of *C. nelsonii* ancestry are shown in maroon and periwinkle, whereas candidate adaptive loci with an excess of *C. nova* ancestry are shown in red and navy. The dashed vertical lines represent the threshold for SNPs that are 2, 2.5, or 3 SD from the mean of the set of inlier loci.
Moreover, adaptive gene flow via hybridization can simultaneously introduce novel alleles at many unlinked loci, potentially promoting rapid evolution by adaptation even when selected traits are polygenic (Abbott et al., 2013; Arnold & Kunte, 2017; Hedrick, 2013). A pool of alleles with potential adaptive value has been argued to be key to the adaptive component to the gene flow (Suarez-Gonzalez et al., 2018).
rapid diversification and/or shifts of lineages into a broad range of niches (e.g., in Darwin’s finches, Lamichhaney et al., 2015; Heliconius butterflies, Kozak et al., 2015; cichlids, Albertson et al., 1999) and, in many cases, adaptive alleles were often moved across species boundaries via hybridization and introgression. Heterozygosity of candidate genes has been implicated in promoting prezygotic reproductive isolation of a hybrid Ostropsis species with its parents (Wang et al., 2021). For more recent hybridization events, studies demonstrate how candidate adaptive loci move between parental species and/or hybrids, potentially facilitating diversification, as in canids (Galaverni et al., 2017), Populus tree species (Chhatre et al., 2018) and Lycaeides butterflies (Gompert et al., 2010). Confirming adaptive introgression by explicitly linking putatively adaptive loci to fitness consequences is challenging (Suarez-Gonzalez et al., 2018). However, analyses of genomic clines offer an alternative approach to identifying introgressed alleles that were probably targets of selection (Gompert & Buerkle, 2012).

In our study, there are multiple lines of evidence that suggest hybridization between two montane sedges involves potentially adaptive alleles at multiple loci. An analytical approach (i.e., the UPG method; Gompert & Buerkle, 2012) identified outlier loci with an excess ancestral contribution of one of the two parental species (Table S4). By itself, this is not sufficient to draw conclusions about the role of these loci, although they require explanation, and differential introgression due to selection is one possibility (among many, including constraints associated with genomic architecture, such as chromosomal re-arrangements; Rieseberg et al., 2003). However, with the complement of the genotype–environment method (RDA) in which we evaluate the potential adaptive role of outlier loci, we confirm that the outlier loci (i.e., loci with an excess ancestry of one of the two parental species) show specific environmental associations while inlier loci (i.e., loci whose ancestry traces to both parental species similarly) do not (Figure 5b). Together, the results make a compelling case that candidate adaptive loci are overwhelmingly associated with hybridization.

Other studies involving recent hybridization have also observed that candidate adaptive loci often have been disproportionately contributed by one of the two parental lineages. For example, the vast majority of loci with excess ancestry and putative adaptive effects on photoperiod and phenology came from one parent in poplar hybrids (Chhatre et al., 2018); a similar pattern was found in pines (Menon et al., 2021), where excess ancestry loci facilitated adaptation associated with freeze tolerance. However, in some species, and even when it is predicted that one parental species will contribute disproportionately to an adaptive shift in the hybrid based on environment data, an excess of ancestry solely from that parental species is not found (e.g., hybridization in sparrows between a salt marsh specialist and a recent salt marsh colonist concluded that introgression of loci associated with salt marsh tolerance surprisingly came from both parents; Walsh et al., 2018). In this regard, it is notable that we found a similar number of outlier loci with excess ancestry from each parent, as have other studies (e.g., Parchman et al., 2013; but see Chhatre et al., 2018). Additionally, the set of candidate adaptive loci traces that ancestry to both parents, in which environmental shifts are associated with precipitation and temperature gradients (Figures 4 and 5). Nevertheless, and regardless of which parent contributed to excess ancestry at candidate adaptive loci, the majority of these loci align with regions outside of the environmental space preferred by C. nelsonii and therefore may be responsible for facilitating an environmental shift in the hybrid (Figures 4 and 5, Table 3; Table S7).

### 4.2 The role of candidate adaptive loci in facilitating environmental shifts in hybrids

Hybrids may occur where parental taxa overlap, or they may be defined by the limit of environmental tolerance of one of the parent species (Kadereit, 2015). Adaptive loci acquired via hybridization represent a potentially important source of genetic variation for adaptation to novel environments (Taylor et al., 2015). For example, hybridization in the sunflower genus Helianthus has been linked to the colonization of environments that are distinct from the parental species, including deserts, salt marshes and sand dunes (Rieseberg, 2006). Likewise, candidate adaptive loci can be traced to hybridization in poplar trees, where the introgression is thought to facilitate adaptation at range limits (Chhatre et al., 2018). Moreover, candidate loci may promote the reproductive isolation of a hybrid from its parents in different ways; for example, hybrid speciation in Ostropsis resulted in a stable hybrid that became reproductively isolated from one parent because of adaptation to iron-rich soils and from the other parent via loci associated with flowering time (Wang et al., 2021). Based on our results, hybridization in montane sedges serves as the source of potential adaptive loci (Figure 5b), and these loci are associated with an environmental shift in which hybrids occupy an environment largely unsuitable for one of the parental species (i.e., C. nelsonii; see Figure 4; Table S7).

Our study also adds to growing evidence within the genus Carex that localized hybridization may be common between species (Cayouette & Catling, 1992; Drury, 1956; Gizaw et al., 2016; Waterway, 1994; Więcław & Wilhelm, 2014). Interestingly, our results highlight how hybridization can provide a source for adaptive divergence even when the hybrids occupy different environmental space (Figure 4) and occur at geographical extremes separated by hundreds of kilometres (Figure 1), as with the comparison of hybrids from northern Colorado/southern Wyoming (i.e., Flat Tops and Medicine Bows) with those from southern Colorado (i.e., Lizard Head, Red Lakes, Mt Oso). This geographical disjunction and differences in environmental space suggests the possibility of independent formation of hybrids (i.e., two separate origins for the hybrid taxon). However, this hypothesis is not supported by our data. For example, hybrids cluster together (with a few exceptions) in phylogenetic analyses (Figure 3; Figure S2), and although the northern hybrids form a clade, the remainder of hybrid individuals do not form distinct clades based on geography (Figure 3; Figure S2). The nesting of a clade of northern hybrids within the grade of hybrids from Lizard Head, Red Lakes and Mt Oso suggests that a founder event...
in the colonization of the north is a more likely explanation for the observed genetic patterns (Figure 3; Figure S2).

Under the hypothesis that hybrids all descend from a single hybridization event, whether the origin occurred in the north vs. the south has important implications for interpreting the genetic and environmental differences between the two hybrid groups. The environmental conditions in the northern hybrids are within the environmental tolerances of C. nelsonii, whereas the environmental conditions experienced by all southern hybrids are well outside of the environmental space occupied by C. nelsonii (Figure 4). Positing a northern origin of hybridization would suggest that the hybridization was a result of overlap of parental ranges, and hence the initial hybridization event reflected a breakdown of reproductive isolation. In contrast, a southern origin would suggest that an adaptive shift facilitated the formation of the hybrid taxon. Both hypotheses are consistent with involvement of candidate adaptive loci in an environmental shift in the hybrid, but the difference is whether the environmental shift was secondary to the formation of the hybrid taxon (i.e., associated with range expansion) vs. the origin of the hybrid taxon. The evidence suggests the latter hypothesis applies here. Specifically, candidate adaptive loci distinguish the hybrid taxon from C. nelsonii, but not C. nova (Figure 5a). This suggests a southern origin for the hybrid taxon that was driven by a shift in the environmental space hybrids occupy away from one parental species—in this case, C. nelsonii (Figure 4). Moreover, when mapped onto the environmental PCA for both parental species and the hybrid, over 80% of candidate adaptive loci align with regions outside of the environmental space preferred by C. nelsonii (i.e., negative values of PC1 and PC2 in the environmental PCA; see Figure 4). We interpret this to mean that although we do not know the precise environmental conditions promoting adaptation in specific candidate adaptive loci, many of the candidate adaptive loci may have increased the tolerance of hybrids to environments unsuitable for C. nelsonii. We note that only a couple of “pure” C. nelsonii individuals have been collected in the southern hybrid region (i.e., in the San Juan mountains) and this is the southern distribution limit of C. nelsonii, whereas C. nova is common. The hybridization event may have allowed the C. nelsonii genotype to persist in this region, albeit as a part of a new, reproductively isolated evolutionary lineage. The San Juan mountains are also known to contain an abundance of endemic montane species (Fowler et al., 2014; Weber, 2003), potentially because the rate of post-glacial climate change was high in the region (Briles et al., 2012). Taxa adapted to colder climates more typical of the more northern Rocky Mountains, such as C. nelsonii, either had to adapt to this new environmental space, or they were extirpated.

4.3 | Caveats with identifying candidate adaptive loci in species with a complex history of hybridization

Hybridization between closely related species can occur in complex spatial and temporal contexts that make it challenging to infer the history of hybridization and interspecific gene flow, even with genomic data. Several analyses point to a complicated history in the focal taxa. For example, the hybrid index and population genetic statistics show the hybrid taxon’s ancestry skews towards C. nova, but other analyses such as STRUCTURE and SVDQUARTETS show a skew towards C. nelsonii (Figures 2 and 3; Figure S2). Backcrossing between some hybrid individuals and a parent may explain some of the observed patterns, especially in the Mt Oso-like hybrids, which show evidence of backcrossing with C. nova in the STRUCTURE and SVDQUARTETS analyses (Figures 2 and 3; Figure S2). Furthermore, we cannot identify the location of outlier loci in the genome due to a lack of a reference genome for these taxa. Consequently, it is possible that genetic drift contributed to the pool of outlier loci, not selection (Gompert & Buerkle, 2011). However, we consider the drift explanation unlikely given that candidate adaptive loci (Table 3) were overwhelmingly outlier loci (Figure 5b). We expected alleles with excess ancestry from one of the other parental species to show an adaptive association with the environmental space occupied by the hybrid. However, neither set of outlier loci showed a significant association with the environment when the hybrids were analysed separately without either parent. This could mean a lack of adaptive importance in the outlier loci, or it could reflect limited statistical power due to the small sample sizes—there were only five hybrid populations. Furthermore, because we used RDAs with population allele frequencies as opposed to individual frequencies to avoid pseudoreplication, a large environmental effect on the loci was needed to detect an association between candidate adaptive genes and local environmental conditions of the hybrid populations.

Processes other than selection may contribute to candidate adaptive loci detected among the outlier loci. The genus Carex has relatively high diversification rates, which are often attributed to chromosomal incompatibilities via holocentric chromosomes (Hipp, 2007). Chromosomal incompatibilities can cause nearly instantaneous speciation, with offspring reproductively incompatible with their parents, but this is an unlikely explanation here because at least one parent (C. nova) was reproductively compatible with the hybrid for some time (Figure 3; Figure S2). Meiotic aberrations are also quite common in Carex and can cause these taxa to behave unlike most other angiosperms, especially with regard to hybridization (Hipp, 2007). There are no chromosome count data available for either of the parental species; counts in the genus range from n = 6–62 (Więcław et al., 2020), limiting our ability to consider the viability of this alternative explanation for the outlier loci. However, it seems peculiar that we observed a similar number of loci with extreme ancestry for each parent; this is consistent with a drift hypothesis, but the environmental association of outlier loci and their position in environmental space makes this unlikely. It may be possible that tight linkage (as with chromosomal rearrangements; see Rieseberg et al., 2003) could underlie the parity of the two parental species’ contribution to outlier loci. For example, if the outlier loci all occur on a single chromosome, some outlier loci may have nothing to do with an adaptive environmental shift in the hybrids. However, not all the outlier loci mapped to
an environmental space distinct from C. nelsonii (Figures 4 and 5b, Table 3). Although we consider the prospect unlikely, we cannot rule out the possibility that the candidate adaptive loci reside on a single chromosome that is inherited as a linkage block, which would suggest that the hundreds of outlier loci we observe may represent many fewer independent loci with excess ancestry from one parent. Because of the documented chromosomal irregularities in Carex, this possibility needs to be considered, even if it is statistically improbable (e.g., if the parental species have only six chromosomes, then >1000 loci would be expected to reside on a single chromosome, and with 607 candidate adaptive loci detected from the outlier loci, it is possible a single inversion could capture all candidate adaptive loci, but that would be a very large linkage block to remain in linkage equilibrium).

5 | CONCLUSIONS

Genomic data considered in light of the geographical distribution of a hybrid taxon, in addition to the environmental space it occupies relative to the parental species, provide complementary evidence that supports the role of hybridization in contributing to an adaptive environmental shift in montane sedges. Our findings corroborate a hybrid taxon morphologically similar to C. nelsonii, but which occupies an environmental space inaccessible to C. nelsonii. Furthermore, given the distinctiveness of the hybrid's environmental distribution compared to the more northerly distributed C. nelsonii, but not the more southerly distributed C. nova, we suggest that the hybridization event, and the concomitant environmental shift, probably originated in the south (perhaps in the San Juan mountains in southern Colorado, where nonadmixed C. nelsonii are rare, but C. nova is common), and hybrids subsequently colonized northern Colorado and southern Wyoming. Several lines of evidence support this historical scenario and suggest that candidate adaptive loci facilitated an environmental shift in the hybrid relative to the parental species C. nelsonii. Our results illustrate how a complementary set of analyses, in addition to independent data sets based on geography and the environment, can provide insight into the potential adaptive role of hybridization even when genomic resources (e.g., whole genome sequences, or a chromosomal scaffold) are lacking and in the absence of quantitative phenotypic data. In other words, the detailed examination of loci within hybrids relative to the genetic backgrounds of parental lineages not only identifies the role of hybridization in the formation of a new lineage of montane sedges, but also shows that hybridization has facilitated an adaptive shift in which hybrid individuals occupy unique environmental conditions.

AUTHOR CONTRIBUTIONS

R.G.J.H. designed the study and performed data analyses. R.M. conceived the study, performed field work and laboratory work, and contributed to data analyses. L.L.K. conceived the study and contributed to data analyses. R.G.J.H. led the writing and all authors contributed to writing and editing the manuscript.

ACKNOWLEDGEMENTS

We thank Giorgia Auteri, Arnaud Becheler, Sasha Bishop, Cecilia Fiorini, and Rachel Wadleigh for valuable discussions that improved the manuscript. We thank Marcella Baiz for assistance in running bgc software and Zach Gompert for providing helpful comments on a draft of the manuscript. Finally, we thank the associate editor and three anonymous reviewers for many valuable comments and suggestions during the review process.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Supporting Information files, including DNA matrices, sampling locations, and GPS coordinates from vetted herbarium specimens are deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.sbcc2fr8f). Scripts for conducting redundancy analyses are available in a GitHub Repository (https://github.com/richiehodel/Carex_hybridization).

ORCID

Richard G. J. Hodel © https://orcid.org/0000-0002-2896-4907

REFERENCES

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., Boughman, J., Breseford, A., Buerkle, C. A., Buggs, R., Butlin, R. K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S. H., Hermansen, J. S., Hewitt, G., Hudson, A. G., Jiggins, C., ... Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology*, 26, 229–246.

Abbott, R. J., Barton, N. H., & Good, J. M. (2016). Genomics of hybridization and its evolutionary consequences. *Molecular Ecology*, 25, 2325–2332.

Albertson, R. C., Markert, J. A., Danley, P. D., & Kocher, T. D. (1999). Phylogenies of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 5107–5110.

Arnold, M. L., & Kunte, K. (2017). Adaptive genetic exchange: A tangled history of admixture and evolutionary innovation. *Trends in Ecology and Evolution*, 32, 601–611.

Arnold, M. L., & Martin, N. H. (2009). Adaptation by introgression. *Journal of Biology*, 8, 82.

Blischak, P. D., Chifman, J., Wolfe, A. D., & Kubatko, L. S. (2018). HyDe: A python package for genome-scale hybridization detection (D posada, Ed). *Systematic Biology*, 67, 821–829.

Briles, C. E., Whitlock, C., & Meltzer, D. J. (2012). Last glacial–interglacial environments in the southern Rocky Mountains, USA and implications for younger Dryas-age human occupation. *Quaternary Research*, 77, 96–103.

Capblancq, T., Luu, K., Blum, M. G. B., & Bazin, E. (2018). Evaluation of redundancy analysis to identify signatures of local adaptation. *Molecular Ecology Resources*, 18, 1223–1233.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, 22, 3124–3140.
Cayouette, J., & Catling, P. M. (1992). Hybridization in the genus Carex with special reference to North America. *The Botanical Review*, 58, 351–438.

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

Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics*, 30, 3317–3324.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., MA, D. P., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., & McVean, G. (2011). The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.

Dion-Côté, A. M., & Barbash, D. A. (2017). Beyond speciation genes: An overview of genome stability in evolution and speciation. *Current Opinion in Genetics and Development*, 47, 17–23.

Dlay, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20.

Drury, W. H. (1956). The ecology of the natural origin of a species of Carex by hybridization. *Rhodora*, 58, 51–72.

Eaton, D. A. R. (2014). PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, 30, 1844–1849.

Eaton, D. A. R., & Overcast, I. (2020). Ipypad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*, 36, 2592–2594.

Fitak, R. R. (2021). OptMe: Estimating the optimal number of migration edges on population trees using Treemix. *Biology Methods and Protocols*, 6, 1–6. https://doi.org/10.1093/biomet/bpab017

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype-environment associations. *Molecular Ecology*, 27, 2215–2233.

Fowler, J. F., Nelson, B. E., & Hartman, R. L. (2014). Vascular plant flora of the alpine zone in the southern Rocky Mountains, USA. *Journal of the Botanical Research Institute of Texas*, 8, 611–636.

Galaverni, M., Caniglia, R., Pagani, L., Fabbri, E., Boattini, A., & Randi, E. (2017). Disentangling timing of admixture, patterns of introgression, and phenotypic indicators in a hybridizing wolf population. *Molecular Biology and Evolution*, 34, 2324–2339.

Gizaw, A., Wondimu, T., Mugizi, T. F., Albers, C. A., Banks, E., MA, D. P., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., & McVean, G. (2011). The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.

Hijmans, R. J. (2017). *dismo*: Species distribution modeling. R package version 1.1-4. https://CRAN.R-project.org/package=dismo

Hijmans, R. J. (2019). *Raster*: Geographic data analysis and modeling. R package version 3.0-2. https://CRAN.R-project.org/package=raster

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.

Hipp, A. L. (2007). Nonuniform processes of chromosome evolution in sedges (Carex: Cyperaceae). *Evolution*, 61, 2175–2194.

Hodel, R. G. J., Massatti, R., Bishop, S. G. D., & Knowles, L. L. (2021). Testing which axes of species differentiation underlie covariance of phylogeographic similarity among montane sedge species. *Evolution*, 75, 349–364.

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070–3071.

Kadereit, J. W. (2015). The geography of hybrid speciation in plants. *Taxon*, 64, 673–687.

Kim, S.-C., & Rieseberg, L. H. (1999). Genetic architecture of species differences in annual sunflowers: Implications for adaptive trait introgression. *Genetics*, 153, 965–977.

Knowles, L. L., & Richards, C. L. (2005). Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology*, 14, 4023–4032.

Kou, Y., Zhang, L., Fan, D., Cheng, S., Li, D., Hodel, R. G. J., & Zhang, Z. (2020). Evolutionary history of a relic conifer, *Pseudotsuga chienii* (Taxaceae), in Southeast China during the late Neogene: Old lineage, young populations. *Annals of Botany*, 125, 105–117.

Kozak, K. M., Wahlberg, N., Neild, A. F. E., Dasmahapatra, K. K., Mallet, J., & Jiggins, C. D. (2015). Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* butterflies. *Systematic Biology*, 64, 505–524.

Kunte, K., Shea, C., Aardema, M. L., Scriber, J. M., Juenger, T. E., Gilbert, L. L., & Kronforst, M. R. (2011). Sex chromosome mosaicism and hybrid speciation among tiger swallowtail butterflies (NA Moran, Ed). *PLoS Genetics*, 7, e1002274.

Lamichhaney, S., Berglund, J., Almén, M. S., Maqbool, K., Grabherr, M., Martínez-Barrio, A., Promerová, M., Rubin, C. J., Wang, C., Zamani, N., Grant, B. R., Grant, P. R., Webster, M. T., & Andersson, L. (2015). Evolution of Darwin’s finches and their beaks revealed by genome sequencing. *Nature*, 518, 371–375.

Leducq, J.-B., Nielly-Thibault, L., Charron, G., Eberlein, C., Verta, J.-P., Samani, P., Sylvester, K., Hittinger, C. T., Bell, G., & Landry, C. R. (2016). Speciation driven by hybridization and chromosomal plasticity in a wild yeast. *Nature Microbiology*, 1(1). https://doi.org/10.1038/nmicrobiol.2015.3

Legendre, P., & Legendre, L. (2012). *Numerical ecology*. Elsevier.

Luu, K., Bazin, E., & Blum, M. G. B. (2017). *Pcadapt*: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, 17, 67–77.

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

Massatti, R., & Knowles, L. L. (2014). Microhabitat differences impact phylogeographic concordance of codistributed species: Genomic evidence in montane sedges (Carex L.) from the Rocky Mountains. *Evolution*, 68, 2833–2846.

Massatti, R., & Knowles, L. L. (2016). Contrasting support for alternative models of genomic variation based on microhabitat preference: Species-specific effects of climate change in alpine sedges. *Molecular Ecology*, 25, 3974–3986.

Massatti, R., & Knowles, L. L. (2020). The historical context of contemporary climatic adaptation: A case study in the climatically dynamic and environmentally complex southwestern United States. *Ecography*, 43, 735–746.

Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017). Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications*, 8, 14363.

Menon, M., Bagley, J. C., Page, G. F. M., Whipple, A. V., Schoettle, A. W., Still, C. J., Wehenkel, C., Waring, K. M., Flores-Renteria, L., Cushman, S. A., & Eckert, A. J. (2021). Adaptive evolution in a
conifer hybrid zone is driven by a mosaic of recently introgressed and background genetic variants. *Communications Biology*, 4, 1–14.

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendeur, P., & McGlinn, D. (2017). Vegan: *Community ecology package*. R package version 2.4-4.

Parchman, T. L., Gompert, Z., Braun, M. J., Brumfield, R. T., McDonald, D. B., Uy, J. A. C., Zhang, G., Jarvis, E. D., Schlinger, B. A., & Buerkle, C. A. (2013). The genomic consequences of adaptive divergence and reproductive isolation between species of manakins. *Molecular Ecology*, 22, 3304–3317.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, 7, e37135.

Pickrell, J. K., & Pritchard, J. K. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genetics*, 8, 1002967. https://doi.org/10.1371/journal.pgen.1002967

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–955.

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, 24, 4348–4370.

Rieseberg, L. H. (1997). Hybrid origins of plant species. *Annual Review of Ecology and Systematics*, 28, 359–389.

Rieseberg, L. H. (2006). Hybrid speciation in wild sunflowers. *Molecular Ecology and Reproductive Isolation between Species of Manakins*. *Molecular Ecology*, 22, 3304–3317.

Rieseberg, L. H. (2007). Plant speciation. *Molecular Ecology*, 16, 3171–3192.

Rieseberg, L. H. (2008). Hybrid speciation in wild sunflowers. *Annals of the Missouri Botanical Garden*, 95, 34–48.

Rieseberg, L. H., Raymond, O., Rosenthal, D. M., Lai, Z., Livingstone, K., Nakazato, T., Durphy, J. L., Schwarzbach, A. E., Donovan, L. A., & Lexer, C. (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*, 301, 1211–1216.

Rieseberg, L. H., & Willis, J. H. (2007). Plant speciation. *Science*, 317, 910–914.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, 19, 198–207.

Suarez-Gonzalez, A., Lexer, C., & Cronk, Q. C. B. (2018). Adaptive introgression: A plant perspective. *Biological Letters*, 14, 20170688.

Taylor, S. A., & Larson, E. L. (2019). Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. *Nature Ecology and Evolution*, 3, 170–177.

Taylor, S. A., Larson, E. L., & Harrison, R. G. (2015). Hybrid zones: Windows on climate change. *Trends in Ecology and Evolution*, 30, 398–406.

Twyford, A. D., & Ennos, R. A. (2012). Next-generation hybridization and introgression. *Heredity*, 108, 179–189.

Vanhove, M., Pina-Martins, F., Coelho, A. C., Branquinho, C., Costa, A., Batista, D., Príncipe, A., Sousa, P., Henriques, A., Marques, I., Belkadi, B., Knowles, L. L., & Paulo, O. S. (2021). Using gradient forest to predict climate response and adaptation in Cork oak. *Journal of Evolutionary Biology*, 34, 910–923.

Walsh, J., Kovach, A. I., Olsen, B. J., Shriver, W. G., & Lovette, I. J. (2018). Bidirectional adaptive introgression between two ecologically divergent sparrow species. *Evolution*, 72, 2076–2089.

Wang, X., Ye, X., Zhao, L., Li, D., Guo, Z., & Zhuang, H. (2017). Genome-wide RAD sequencing data provide unprecedented resolution of the phylogeny of temperate bamboos (Poaceae: Bambusoideae). *Scientific Reports*, 7, 1–11.

Wang, Z., Jiang, Y., Bi, H., Lu, Z., Ma, Y., Yang, X., Chen, N., Tian, B., Liu, B., Mao, X., Ma, T., DiFazio, S. P., Hu, Q., Abbott, R. J., & Liu, J. (2021). Hybrid speciation via inheritance of alternate alleles of parental isolating genes. *Molecular Plant*, 14, 208–222.

Waterway, M. J. (1994). Evidence for the hybrid origin of Carex knieskernii with comments on hybridization in the genus Carex (Cyperaceae). *Canadian Journal of Botany*, 72, 860–871.

Weber, W. A. (2003). The middle Asian element in the southern Rocky Mountain flora of the western United States: A critical biogeographical review. *Journal of Biogeography*, 30, 649–685.

Whitney, K. D., Randell, R. A., & Rieseberg, L. H. (2010). Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. New Phytologist, 187, 230–239.

Wienclaw, H., Kalkinka, A., & Koopman, J. (2020). Chromosome numbers of Carex (Cyperaceae) and their taxonomic implications. *PLoS One*, 15, e0228353.

Wienclaw, H., & Wilhelm, M. (2014). Natural hybridization within the *Carex flava* complex (Cyperaceae) in Poland: Morphometric studies. *Anales Botanici Fenici*, 51, 129–147.

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, 28, 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

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Additional supporting information may be found in the online version of the article at the publisher’s website.