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Complete List of Authors:	McVay, John; Duke University Department of Biology Hauser, Duncan; Duke University Department of Biology HIPPI, ANDREW; The Morton Arboretum, ; The Field Museum, Botany Manos, Paul; Duke University Department of Biology
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Phylogenomics reveals a complex evolutionary history of lobed-leaf white oaks in
Western North America

Authors

John D McVay¹

Duncan Hauser¹

Andrew L. Hipp²

Paul Manos¹

ABSTRACT

Quercus (oak) species hybridize in complex patterns that have yet to be fully explored with phylogenomic data. Analyses to date have recovered reasonable divergent patterns, suggesting that the impact of introgression may not always be obvious in inferred oak phylogenies. We explore this phenomenon using RADseq data for 136 samples representing 54 oak species by conducting phylogenetic analyses designed to distinguish signals of lineage diversification and hybridization, focusing on the lobed-leaf species *Quercus gambelii*, *Q. lobata*, and *Q. garryana* in the context of a broad sampling of allied white oaks (*Quercus* section *Quercus*), and particularly the Midwestern *Q. macrocarpa*. We demonstrate that historical introgressive hybridization between once sympatric species affects phylogeny estimation. Historical range expansion during periods of favorable climate likely explains our observations; analyses support genetic exchange between ancestral populations of *Q. gambelii* and *Q. macrocarpa*. We conclude that the genomic consequences of introgression caused the attraction of distant lineages in phylogenetic tree space, and that introgressive and divergent signals can be disentangled to produce a robust estimate of the phylogenetic history of the species.

Keywords: *Quercus*, RADseq, hybridization, introgression

INTRODUCTION

The advent of second generation DNA sequencing technologies has lent unprecedented power to genetic and genomic analyses across life science disciplines. As phylogenetic tools have been developed to accommodate these datasets, patterns of lineage divergence and diversification have emerged that were previously undetectable (e.g., O'Neill et al. 2013; Piednoël et al. 2012; Prum et al. 2015). And though historical hybridization has remained a challenge to model in a phylogenetic framework (Nakhleh 2013), the scale of datasets afforded by these new technologies has allowed for more robust perspectives on the role that introgression may play in shaping the tree of life (Arnold 2015; Baptiste et al. 2013). Oaks, having long been suspected to hybridize widely (and confirmed genetically, in many cases; e.g., Belahbib et al. 2001; Craft et al. 2002; Eaton et al. 2015; Kremer et al. 2002; Zeng et al. 2010), are an ideal system in which to apply these novel approaches to investigating reticulating evolutionary patterns.

Recent studies using nuclear DNA markers suggest that current hybridization will not pose an insurmountable barrier to understanding the shape and timing of the oak phylogeny (Eaton et al. 2015; Hipp 2015; Hipp et al. 2014; Muir et al. 2000; Pearse and Hipp 2009). Moreover, multigene datasets provide evidence for the existence of species boundaries among relatively closely related species occurring in sympatry, even in the face of gene flow (e.g., Cavender-Bares et al. 2015; Craft et al. 2002; Eaton et al. 2015; Gailing and Curtu 2014; Hipp and Weber 2008; Lepais and Bacles 2014; Leroy et al. 2017). However, it is undoubtedly the case from these and related studies that oaks have

been hybridizing since their inception, and the signature of historical introgression in the oaks may be particularly cryptic, because signals of ancient introgression are effaced by the more obvious signals of contemporary gene flow. Genetic evidence suggests the presence of several former contact zones where only traces of the alleles (Dodd and Afzal-Rafii 2004) or morphology (Maze 1968) of a contributing parental species can be found today. But we lack a phylogenetic perspective on these ancestral hybridization stories. Such a perspective is needed to distinguish contemporary hybridization between modern species from historical gene flow among interior branches of the phylogeny.

The white oaks of North America, represented by more than 50 species and several discrete syngamea that have been noted in the literature (Grant 1981; Hardin 1975), offer a particularly useful system for investigating the interplay between contemporary and historical hybridization in generating the diversity we observe in oaks today. Preliminary phylogenomic data suggest that biogeographic boundaries and differential climatic regions have shaped the evolution of regionally limited clades (Manos 2016). The relationship between eastern and western white oak species is interesting in this regard, particularly the species with lobed leaves (Fig. 1). Lobed-leaf species are concentrated in eastern North America and best exemplified by the most common species, *Q. alba*. However, the ranges of several other eastern lobed-leaf species extend west into the Dakotas and Texas (e.g. *Q. macrocarpa*). In western North America, two lobed-leaf species, *Q. lobata* and *Q. garryana*, are typical of more mesic California Floristic Province (CFP) habitats. Of long standing interest is *Q. gambelii*, a shrub to small tree

common throughout the southern Rocky Mountains, but fragmented in distribution (see Fig. 1). Despite the high degree of leaf and trichome similarity among *Q. gambelii*, *Q. lobata* and *Q. garryana* (e.g., Nixon and Muller 1997), *Q. gambelii* has never been classified with other lobed-leaf species (Camus 1936-1954; Muller 1951; Trelease 1924). *Quercus gambelii* is also known to hybridize with at least 7 species of white oaks in the southwestern US (Howard et al. 1997; Tucker 1963). Given its distribution intermediate between the ranges of eastern and western lobed-leaf clades, the phylogenetic position of *Q. gambelii* remains an important unsettled question in oak systematics and biogeography.

In this paper, we use genomic data to address the phylogeny of the lobed-leaved white oaks of western North America and to investigate hypotheses of introgressive hybridization. We utilize a reduced-representation genomic sequencing approach, restriction-site associated DNA sequencing (RADseq), combined with phylogenetic analyses that partition the dataset to distinguish primary and secondary phylogenetic signals, reflecting both lineage diversification and hybridization, that have shaped the origins of *Quercus gambelii*, *Q. lobata*, *Q. garryana* and allied species. In the end, we present the first phylogenetic history of the interconnections between western and eastern North American oak clades that today constitute distinct evolutionary lineages.

METHODS

Sample collection.—We sampled 134 individuals of *Quercus* representing 53 species of white oak, *Q. pontica* and *Q. sadleriana*, two species from subsection *Virentes* (all representing § *Quercus*), and three species of intermediate oaks (§ *Protobalanus*), and two individuals of *Notholithocarpus densiflorus* as an outgroup (Appendix). Within the North American (NA) white oaks, we sampled individuals from each provisionally named subclade identified in preliminary analyses, including *Albae*, (Eastern NA), *Dumosae* (California, USA), *Prinoideae* (Eastern-Midwestern NA), *Stellatae* (Eastern NA), and two unnamed groups whose ranges span Southwestern NA and California, and Mexico and Central America. Our sampling was especially rich in the lobed oaks of the West and Southwest United States, including ten *Quercus gambelii* from populations throughout its range (Fig 1.), five *Q. garryana* and three *Q. lobata* (*Dumosae*) and nine *Q. macrocarpa* (*Prinoideae*; Fig. 1). DNA was extracted from fresh or frozen leaf tissue using the DNEasy plant mini kit (Qiagen, Hilden, Germany) or a modification of the CTAB method optimized for hardwood trees by Lefort and Douglas (1999; supplemental materials). Restriction site-associated DNA (RAD) library preparations were performed by Floragenex (Portland, OR, USA), using the *Pst*I restriction enzyme, as described previously (Baird et al. 2008; see additional discussion in Hipp et al. 2014). Sequencing was conducted between 2012 and 2016 on an Illumina Genome Analyzer IIx at Floragenex or an Illumina HiSeq 2500 at University of Oregon.

Analysis of RADseq.—We processed raw sequence data in pyRAD 3.066 (Eaton 2014), discarding low quality raw reads with more than four nucleotides with a Phred 33 score below 20. Loci were clustered within (at a minimum copy depth of six) then among

samples (minimum of four individuals per locus) with a minimum 0.88 sequence similarity threshold.

Phylogeny estimation.—We performed a maximum likelihood analysis with 100 non-parametric bootstraps of the concatenated pyRAD output using RAxML 8.2.9 (Stamatakis 2014), under the GTRCAT approximation of the general time-reversible nucleotide substitution model with rate heterogeneity (GTR+gamma) (Stamatakis 2006). Because concatenated data analysis does not model the processes underlying gene tree discordance, we estimated a coalescent species tree using the SVDquartets method (Chifman and Kubatko 2014, 2015) implemented in PAUP (Swofford 2003; Swofford 2016). We choose this method over other available methods that rely on summary statistics as input (e.g.; Mirarab et al. 2014), as any singular gene tree (estimated from ~85bp sequence data) is likely to be a poor estimate of the underlying genealogy. We performed an exhaustive quartet analysis (i.e., all possible quartets that include a representative of four species were sampled) under the species tree model and performed 100 bootstrap replicates to assess support across the data of the point estimate.

Rank of relatedness based on summed evolutionary distance.—The topology observed in a concatenated phylogenetic analysis may be the product of a single underlying phylogenetic signal; alternatively, when conflicting signal is present, particularly due to introgression, affected lineages may be placed in a position intermediate to their expected placement were no introgression to have occurred (McDade 1992). This result may be particularly problematic when interrogating large datasets, as bootstrap support may be

strong despite the presence of phylogenetically conflicting signal (Phillips et al. 2004). Therefore, we interrogated the data set on a locus-by-locus, sample-by-sample basis, to assess several points of genealogical discordance among loci. For each individual in the dataset we ranked relatedness to all other individuals in the dataset based on the sum of evolutionary distance estimated from each locus. For each locus, we produced a pairwise distance matrix of HKY-modeled (Hasegawa et al. 1985) neighbor joining distances calculated in PAUP. Then we sorted the distances numerically for each individual. All individuals that exhibited the shortest pairwise distance to the targeted individual were awarded the minimum evolution score of the locus; individuals not meeting this criterion were awarded a score of zero. These scores were then summed for each non-target individual across all loci, scaled by the number of loci each shared with the target individual, then ranked; here we consider the highest scoring individuals considered as being most closely related to the targeted individual. In a purely divergent species tree (i.e., with a single underlying phylogenetic signal), our expectations are that ranks would most closely reflect the branching order of the phylogenetic estimate. To narrow the focus of the analysis, we chose one individual each for *Q. gambelii*, *Q. lobata*, and *Q. macrocarpa*, and calculated the rankings for all other individuals in the dataset, paying particular attention to the rankings of *Q. macrocarpa* samples in relation to the *Q. gambelii* and *Q. lobata* samples, and the rankings of the *Q. gambelii* samples in relation to the *Q. macrocarpa* sample. As a null, we observed the rankings of *Q. michauxii* (an Eastern NA oak not in contact, nor suspected to interact with any of the western lobed oaks) specimens to the *Q. macrocarpa* individual.

Deviations from expected rankings may be caused by gene tree-species tree conflict due to incomplete lineage sorting (ILS) rather than, or in addition to introgression. To assess how these rankings compare to our expectations in the presence of varying degrees of incomplete lineage sorting (ILS), we simulated and analyzed datasets based on the total evidence concatenated estimate with branch lengths modified to simulate three coalescent depths: 1, 2.5, and 10 N-generations, representing differing degrees of expected discordance among gene trees. We simulated 500 datasets at each depth; for each simulated depth, we employed Mesquite (Maddison and Maddison 2011) to simulate $1e5$ coalescent gene trees contained within the concatenated estimate with branch lengths modified to the desired three depth. Then, for each simulation replicate, we simulated sequence data on a randomly chosen simulated gene tree, with taxon sampling drawn randomly from the subset of loci in the data in which one or more of the three targeted individuals were represented. We drew from the distribution of taxon sampling for the real dataset to simulate both the degree and non-random nature of missing data. We used seq-gen (Rambaut and Grassly 1997) to simulate 85-basepair clusters for each with substitution rate (tree length) for each cluster drawn from a uniform distribution (0.001-0.02). Each simulated dataset was then analyzed in the manner described for the real dataset. To assess significance of the observed rankings, we calculated a test statistic as the arithmetic mean of rank of a targeted taxon (e.g., the mean rank of relatedness of all *Quercus macrocarpa* samples with respect to the *Q. gambelii* sample); this value was compared to a distribution of mean ranks of the same individuals across all simulated datasets for each depth. We were interested in individuals more closely related to the queried individuals, significance was assessed as a one-tailed test, where a p-value is the

proportion of simulated mean ranks that were higher than the observed value, divided by the number of simulations (500).

Because inclusion of hybrid individuals can have incompletely predictable effects on phylogenetic inferences across the tree, we sought to identify individuals of *Q. gambelii*, *Q. lobata* and *Q. macrocarpa* that were of potential hybrid ancestry. The 22 samples of these three species were re-clustered via pyRAD, then we analyzed all 22 samples using the admixture model implemented in *Structure* version 2.3.4 (Pritchard et al. 2000), as has been done in other interspecific oak studies (Antonecchia et al. 2015; Eaton et al. 2015; Lind and Gailing 2013). Two datasets were created. The first sought to minimize missing data by only including loci that were shared between at least 70% of the samples (average of 10.45% missing data) following Eaton et al. (2015). The second dataset comprised all loci shared among a minimum of four individuals in the pyRAD output (average of 39.2% missing data). For each dataset, five replicates were run from $K=2$ to $K=5$ using default settings with 20k burn-in steps (during which F_{ST} and α sampling stabilized) followed by 20k MCMC steps. Any individuals that were determined to be potentially admixed (<95% any single species) were removed, and we estimated the maximum likelihood phylogeny for the remaining samples following the methods described above.

Assessment of hybridization using Patterson's D.—In order to assess evidence of hybridization between between three species of lobed-leaf oaks, we employed Patterson's D statistic (Durand et al. 2011; Green et al. 2010) which tests for significant deviation

from expected proportions of shared biallelic SNPs that are discordant with the species tree (“ABBA-BABA”), which occur in equal frequencies on average between neutrally evolving populations without gene flow. We implemented a four taxon test in PyRAD, using the pectinate topology (((P1,P2)P3)O), where P1 = *Quercus lobata*, P2 = *Q. gambelii*, P3 = *Q. macrocarpa*, and O (outgroup) = *Q. pontica*. We chose *Q. pontica* for the outgroup as it branches earlier relative to the ingroup, and is unlikely to have overlapped in historic range with any of the ingroup taxa. We evaluated all possible quartets containing one of eight *Q. gambelii*, three *Q. lobata*, and nine *Q. macrocarpa* for a total of 216 comparisons. One thousand bootstrap replicates were performed for each comparison, and D-statistics were considered significant if their z-score corresponded to $p < 0.01$ after Holm-Bonferroni correction for multiple comparisons.

Minimizing the signal of Q. gambelii-macrocarpa introgression.—In initial ranking relatedness analyses, *Quercus macrocarpa* samples exhibited the highest non-conspecific relatedness value for individuals of *Q. gambelii*, a relationship inconsistent with both the concatenated and species tree analyses. Because of these observations and the proposed historical interaction between these two species (Maze 1968), we sought to remove potential signal of introgression between *Q. gambelii* and *Q. macrocarpa* via a modified reverse successive weighting approach (Trueman 1998) based on decay of likelihood scores when constraints are enforced. Our approach focused on loci for which the monophyly of *Q. gambelii* and *Q. macrocarpa* was a poor fit. For each locus that contained at a minimum one *Q. gambelii*, one *Q. macrocarpa* and two oaks of any other species (filtered this way such that our constraint would not be compatible with all

possible topological outcomes), we calculated likelihood scores on two neighbor-joining trees: one unconstrained and one in which monophyly of the two species in focus was enforced. All loci for which the likelihood was worse when constrained were concatenated and a maximum likelihood phylogeny was estimated in RAxML under the same parameters used for the total evidence estimate. To further test whether interactions between *Q. macrocarpa* and both *Q. gambelii* and *Q. lobata* were affecting the topology in total evidence analysis, we created and analyzed, in the same manner described above, a subsample comprising loci for which the monophyly of *Q. gambelii* and *Q. lobata* was a poor fit. If both species or the ancestor to both species interacted historically with *Q. macrocarpa*, our expectation is that analysis of this subsample would recover *Q. gambelii* and *Q. lobata* as aligned with eastern clades, though no longer in a sister relationship with each other.

RESULTS

Sequencing results.—RAD sequencing of 136 individuals resulted in 2.11e8 total raw reads of which 1.89e8 passed quality filtering (per individual mean = 1.40e6; range 1.08e4-4.68e6, sd = 7.73e5). Fastq files are deposited in the Genbank sequence read archive (SRA project PRJNA376740). The pyRAD-clustered dataset consisted of 86,022 total clusters shared among a minimum of four individuals (approximately exponentially distributed; mean = 20.67, range = 4-126, 25th -75th quartiles = 5-23) individuals per cluster; see supplemental information). The three individuals with the poorest

representation in the final dataset (represented by fewer than 200 clusters) were removed from analyses.

Phylogenetic analysis and Structure results.—The maximum likelihood phylogeny estimated in RAxML (Fig. 2) Within section *Quercus* (excluding *Virentes*, *Protobalanus* and *Q. sadleriana*), we recovered strongly supported clades of California white oaks (*Dumosae*), two Mexican clades, and three Eastern North American clades (*Albae*, *Prinoideae* and *Stellatae*). Samples of *Quercus gambelii* and *Q. lobata* were recovered as each other's closest relatives, composing a clade that was sister to *Albae* and *Prinoideae*. The concatenated estimate is largely topologically concordant with the SVD quartets-based species tree estimate (where the trees can be compared; OTUs are not identical), with the exception of the poorly supported placements of *Q. gambelii* and *Q. lobata*, with the former falling sister to a clade comprising *Albae* and *Prinoideae*, and the latter being sister to all white oaks to the exclusion of *Ponticae* and *Virentes* (supplement). Structure analysis detected three potentially admixed individuals among the three species examined: one *Q. gambelii* from Colorado (~15% *Q. macrocarpa*; our easternmost sample) and two *Q. gambelii* from Utah (~10-15% *Q. lobata*). To eliminate possible topological effects of these individuals, we removed them from the data matrix and re-estimated the phylogeny via RAxML. The resulting topology (not shown) exhibited the same clade-level topology as that of the total evidence tree, suggesting that these putatively admixed individuals were not solely responsible for the observed conflict.

Ranking relatedness.—For each individual in the dataset, we ranked relatedness of all other individuals based on an aggregation of shortest pairwise distances for each locus; this was completed for the collected dataset and three simulated datasets. Here we focused our assessment of these results on samples of three taxa: *Q. gambelii*, *Q. lobata*, and *Q. macrocarpa* (Fig. 3; see supplemental for complete scores).

Individuals of *Q. gambelii* that were represented by more than 500 loci in the dataset ranked as each other's closest relatives in all but a few cases (Fig 3A). The highest ranking non-conspecific sample to any *Q. gambelii* was an individual of *Q. macrocarpa*; all *Q. macrocarpa* individuals were typically highly ranked, with a few individuals from the other clades attaining a higher score for some individuals of *Q. gambelii* (e.g., Fig 3A.). Mexican Clade 2 samples composed most of the next tier of ranked individuals for all samples of *Q. gambelii*. *Quercus lobata* specimens, which are recovered as sister to *Q. gambelii* in the concatenated analysis typically were ranked lower than expected for all individuals. In assessing the significance of rankings for this individual, we focused on the mean rank of *Q. macrocarpa* specimens (Fig. 3A). Observed rankings were significantly higher ($p < 0.002$) than the simulated means for all tree depths, with no single simulated mean rank higher (closer to target individual) than the observed value.

For the three samples of *Q. lobata*, conspecifics ranked highest, followed by *Q. gambelii* and *Q. macrocarpa* (Fig. 3B). This pattern is inconsistent with the rankings observed in the other two species, where *Q. lobata* was not recovered as a highly ranked taxon. Observed rankings of *Q. macrocarpa* specimens to the *Q. lobata* individual were

significantly higher than the simulated means for all tree depths, with no single simulated mean rank higher than the observed value (Fig. 3B).

Individuals of *Q. macrocarpa* (Bur oak) all ranked as each other's closest relatives, followed by *Q. gambelii*, consistent with the ranking of the latter (Fig. 3C). Ranked next highest were typically a mixture of samples representing Prinoideae and Mexican Clade 2, with *Q. lobata* samples on the lower end. The observed mean rank of *Q. gambelii* samples was significantly higher than simulated mean ranks at all tree depths (Fig. 3C). For the null comparison, we found that observed mean rank of *Q. michauxii* samples to the *Q. macrocarpa* samples was significantly lower than simulated mean ranks in two of three depths, with the observed value being greater than three of 500 simulated values ($p = 3/500 \times 12 [\text{Holm-Bonferroni correction}] = 0.072$). Our expectations were that the null observed value would fall within the range of the simulated distributions. The lower-than-expected value is likely due to interacting taxa (like *Q. gambelii*) "pushing" *Q. michauxii* lower in the rankings.

Detecting hybridization with Patterson's D.—Of 216 four-taxon tests, 102 had significant z-scores ($> |4.05|$; see summary in Table 1, full results in supplemental material). Six of eight *Q. gambelii* individuals exhibited both significant positive and negative D-statistics. This phenomenon is likely explained by the narrow overlap of the loci shared among individuals across permutations of the four-taxon test.

Minimizing the Q. macrocarpa-gambelii interaction.—We estimated a maximum likelihood tree for loci whose substitution patterns were incompatible with a sister relationship between *Q. gambelii* and *Q. macrocarpa* (Fig. 4A). 24,712 of 86,022 passed the initial filtering step for loci that would be potentially informative based on taxon sampling; of these 8,636 had a lower likelihood when monophyly of *Q. gambelii* - *Q. macrocarpa* was enforced. The topology of the subsampled dataset differed from the concatenated topology at two deeper nodes: while *Q. lobata* and *Q. gambelii* remained sister (but with poor support), this clade was supported (b.s. = 92) as sister to Dumosae. This clade together was recovered as sister to Stellatae and the Mexican clades, whereas it is placed as sister to all section *Quercus* clades in both the total evidence phylogenetic and species tree estimate. For the second subsampled analysis, in which we filtered loci for those which rejected the monophyly of *Q. gambelii* and *Q. lobata* (4,262 of 15,561), the latter was recovered as sister to the CFP clade, while *Q. gambelii* was recovered as sister to the clade comprising *Q. bicolor*, *Q. lyrata* and *Q. macrocarpa* (Fig. 4B).

DISCUSSION

Our first total evidence (concatenated) maximum likelihood analysis suggested that a clade comprising *Q. gambelii* and *Q. lobata* was sister to the Eastern North American (ENA) lobed white oaks, and more distantly related to the white oaks of the California Floristic Province (CFP). Though strongly supported, this result was only recovered when both *Q. gambelii* and *Q. macrocarpa* were analyzed in combination, suggesting that the result was an artifact of taxon sampling. Based on the additional locus-

partitioning analyses presented in this paper, we consider this total-evidence topology to be misleading: the primary signal of lineage divergence is overwhelmed in this topology by a history of introgression among *Q. gambelii*, *Q. lobata*, and *Q. macrocarpa*. This conclusion is supported by four lines of evidence: sensitivity of the topology to taxon sampling (as mentioned above); rank of relatedness among individuals and four-taxon test, which demonstrate a significantly high degree of allele sharing between *Q. gambelii* and *Q. macrocarpa*; and the strongly supported secondary signal of monophyly of the CFP oaks, when loci supporting the primary topology are removed. We interpret these results with caution, but conclude that the placement of the *Q. gambelii*-*Q. lobata* lineage in the concatenated estimate as sister to eastern oak clades is likely the result of shared, introgressed alleles between *Q. gambelii*, and possibly *Q. lobata*, and *Q. macrocarpa*, “pulling” the western species away from the CFP oaks and towards the ENA clades.

Our inference that our phylogenetic results are due to historical rather than contemporary gene flow is supported by the fact that *Q. gambelii*, *Q. macrocarpa*, and *Q. lobata* are strongly monophyletic across analyses. All individuals of these species form tight, cohesive clades, and all individuals of each species shows the same pattern in rank of relatedness among species with little exception. The one pairwise comparison in which we observed an interruption of a species (Fig 3A) in rank order may be the result of more recent introgression with Mexican oaks along the lineage history of the aberrant sample (*Q. gambelii* sample PM237 from New Mexico, where it is sympatric with Mexican oak species; see supplement). In cases of regional or more local gene flow, introgressed individuals generally fall sister to a core of the species, and their removal is expected to

render the species monophyletic (Eaton et al. 2015; Eaton and Ree 2013; McDade 1990; McDade 1992). Our data show no such pattern. Moreover, Structure analysis shows very little admixture in our study: we would expect to find evidence of admixture at the individual level if our results were the outcome of contemporary gene flow. What admixture is seen may be difficult to interpret, owing to unknown interactions with Mexican clade 2 individuals that are not sampled in this study. The four-taxon test performed using Patterson's D statistic resulted in strong evidence for gene flow between *Q. macrocarpa* and both *Q. lobata* and *Q. gambelii*, however results of permutations that contained the same individual *Q. gambelii* exhibited strongly supported conflicting patterns of gene flow (i.e., ABBA vs BABA); overall six of eight individuals showed this pattern. These discordant patterns may arise from the lack of connectivity, or "sharedness", of loci across four-taxon permutations. Additionally, our particular case made it difficult to set up the test, given that 1) the underlying species tree is not clear, and 2) the outgroup was difficult to choose, due both to the first problem, as well as potential interactions of outgroup candidates that overlap in range, or are thought to have historically shared habitat. Our outgroup choice, *Q. pontica*, is distantly related to the ingroup taxa, an attribute which may lead to false positive detection of gene flow in some cases (Eaton and Ree 2013). Our rank of relatedness approach offers a more global perspective of conflicting signal across the dataset; however, tests of significance should be targeted to a single individual to avoid overestimating significance of outcomes while performing multiple comparisons.

It is worth noting that despite *Q. gambelii*'s reputation for hybridization with a diverse assemblage of white oak species distributed in the southwestern U.S. (Tucker 1961), we found little evidence of gene flow between *Q. gambelii* and those species sampled in this study. The *Q. undulata* complex is thought to entail a syngameon among *Q. arizonica*, *Q. grisea*, *Q. havardii*, *Q. mohriana*, *Q. muehlenbergii*, and *Q. turbinella*, with *Q. gambelii* at the center of these interactions (Tucker 1961). We included at least one individual of each of these species in our analysis, and none appear to share more alleles with *Q. gambelii* than expected by their phylogenetic position, based on the rank of relatedness analyses (supplement). Moreover, *Q. gambelii* exhibited no phylogenetic movement toward species in the Mexican oak clades when secondary topologies (using reverse successive weighting) were estimated (Fig 4). Interestingly, the Mexican samples were clustered near the top of the relatedness rankings among the three species in focus, which was not expected under any simulations of ILS (supplement). These rankings are not surprising given the degree to which these species overlap; in particular *Q. gambelii* is currently sympatric or at least geographically proximal to members of this clade. These results most likely reflect contemporary or recent gene flow, but additional sampling is needed to investigate this pattern. Surprisingly, the lobed-leaf *Q. garryana* aligned more consistently with California white oak species (including members of *Dumosae* and Mexican Clade 2) than with the other lobed western oaks. This may be due to limited interactions between *Q. garryana* and *Q. lobata*, whose ranges overlap narrowly.

While most studies of oak hybridization have documented current gene flow among sympatric species, there have been a few claims of historical secondary contact between species that are now distributed allopatrically (Eaton et al. 2015; Maze 1968).

Macrofossil evidence is clear on how climate change has shaped oak distributions through the last 15 million years (for review see Betancourt et al. 1990; Graham 1999). Leaf fossils from Late Miocene to the Pliocene show that lobed-leaf white oaks (e.g., *Q. prelobata*) had a distribution further north, with a notable density in the region of east central Oregon (e.g., John Day fossil beds), and into southeastern Washington and southwestern Idaho, essentially in areas that no longer harbor oaks (for review see Mensing 2015). Our results are consistent with a broad contact zone between ancestral populations *Q. gambelii* and *Q. macrocarpa* (Fig. 5). However, it's also possible that *Q. gambelii* is a stabilized species of hybrid origin between disjunct populations of a taxon like *Q. prelobata* and *Q. macrocarpa* that has become homogenized through time. While distinguishing between these two scenarios may be difficult with RADseq data, it would not be surprising to discover an oak species derived through hybridization. Interestingly, hypotheses of past hybridization between *Q. gambelii* and *Q. macrocarpa* during the Pleistocene, when oak distribution expanded again, have been used to explain intermediate phenotypes in western North Dakota and northeastern New Mexico, areas where only one of the species occur today (Maze 1968). Based on the lack of evidence suggesting direct interactions between *Q. macrocarpa* and *Q. lobata*, we reject that observed topology is the result of historical exchange between ancestral populations of *Q. macrocarpa* and the ancestor of *Q. gambelii* and *Q. lobata*, though we recognize that this scenario is not incompatible if subsequent exchange between *Q. lobata* and other white oaks in the CFP obscured the primary phylogenetic signal. We observed some evidence for recent admixture between *Q. gambelii* and both *Q. macrocarpa* and *Q. lobata*, in samples collected from the eastern and western edges of the *Q. gambelii* range. In sum,

there may have been at least two temporally distinct rounds of introgression affecting the phylogenetic position of the *Q. gambelii* and *Q. lobata* lineage.

Behavior of hybrids in a phylogenetic analysis. The discovery of hybrids in a phylogenetic context has often been a happy accident of observed incongruence among the markers selected (e.g., Rieseberg and Soltis 1991). With the high volumes of data we now obtain in phylogenomic datasets, the need to carefully subset loci and investigate secondary signals demands attention to weakly supported or unexpected resolutions in the phylogeny. At the risk of fishing for interesting results, phylogeneticists need to pay attention to possible areas of discordance in their phylogenies and begin to investigate whether first impressions that something is amiss are actually born of genealogical discordance. In any case where historical hybridization is suspected, application of a bifurcating model of evolution is not an ideal approach. Network-based phylogenetic analyses seek to accommodate these processes (Nakhleh et al. 2005; Yu et al. 2014; Yu and Nakhleh 2015; Yu et al. 2013), however the potential myriad of genetic exchanges among sympatric oak species across time and space makes the task of modeling these reticulation events daunting and currently intractable. Yet, the concatenated approach and the targeted downstream analyses of relationships presented here complement other SNP-based methods (Durand et al. 2011; Eaton et al. 2015; Eaton and Ree 2013; Green et al. 2010; Pease and Hahn 2015) and provide a framework for tackling this task, leading to more pointed and potentially more powerful hypothesis testing, particularly as we collect more robust genomic resources (transcriptomes, whole genomes) for these taxa (Payseur and Rieseberg 2016).

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Draft

Table 1. Results of four-taxon test of Patterson’s D-statistics of species tree topology (((P1,P2)P3)O), where P1 = *Quercus lobata*, P2 = *Q. gambelii*, P3 = *Q. macrocarpa*, and O = *Q. pontica*. Shown are *Q. gambelii* specimens. Value of third and fourth columns show number of tests (out of 27 total for each individual) that showed significantly positive (ABBA) and negative (BABA) D-scores, respectively.

Table 1.

Introgressed with <i>Q. macrocarpa</i> :			
Sample	State	<i>Q. gambelii</i>	<i>Q. lobata</i>
MOR_387	Colorado	13	2
MOR_546	Arizona	0	15
PM_235	New Mexico	0	7
PM_236	New Mexico	9	6
PM_237	New Mexico	4	6
PM_238	New Mexico	10	5
PM_241	Utah	5	7
PM_242	Utah	8	4

Figure 1. Range map and leaf silhouettes of targeted white oaks species in southwestern North America and the California Floristic Province. Species range shape files downloaded from Little's Atlas of United States Trees (<http://gec.cr.usgs.gov/data/little/>).

Figure 2. Maximum likelihood phylogenetic estimate of the complete concatenated RADseq dataset. Colored nodes correspond with species and clade identities presented on map and in other figures.

Figure 3. Observed and simulated ranks of relatedness for A) *Quercus gambelii* specimen MOR 387 from Colorado, USA; B) *Q. lobata* specimen MOR_123 from California, USA, and C) and D) *Q. macrocarpa* specimen MOR 356 from Illinois, USA. The left panel of each test represents the ranks, from top to bottom of the observed data, with bar colors representing species. The right panel shows box mean ranks of targeted species, indicated above observed statistic (= red) line, of 500 datasets simulated at three coalescent depths (N-generations).

Figure 4. Modified depiction of maximum likelihood phylogenetic estimates of subsamples that reject monophyly of A) *Q. macrocarpa* and *Q. gambelii* and B) *Q. gambelii* and *Q. lobata*; C) collapsed topology of unconstrained estimate. Circles at nodes represent bootstrap support greater than 70%. Subclades sizes and branch lengths have been modified for simplicity and are not to scale.

Figure 5. Plausible scenarios for historical genetic exchange among three oak species across time. 1) Recent interactions between *Q. lobata* and *Q. gambelii* via range expansions during glacial cycles; 2) Recent and historical interactions between *Q. gambelii* or 3) ancestor to *Q. gambelii* and *Q. lobata* (possibly *Q. prelobata*), and *Q. macrocarpa* when during periods of range overlap, and 4) a less likely scenario of a stabilized hybrid formed between historical ancestral populations of *Q. lobata* ancestor and *Q. macrocarpa*. Fading of deepest branches indicates that these are not sister clades.

Draft

222x176mm (300 x 300 DPI)

A. *Q. gambelii*

B. *Q. lobata*

C. *Q. macrocarpa* - *Q. gambelii*

D. *Q. macrocarpa* - *Q. michauxii*

A

No "*Q. gambelii* + *Q. macrocarpa*"

B

No "*Q. gambelii* + *Q. lobata*"

C

Unconstrained