



27 **Abstract**

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29 Hybridization and introgression are common processes among numerous plant species that  
30 present both challenges and opportunities for studies of species delimitation, phylogenetics,  
31 taxonomy, and adaptation. *Rhus integrifolia* and *R. ovata* are two ecologically important shrubs  
32 native to the southwestern USA and Mexico, and are known to hybridize frequently, but the  
33 morphological, genetic, and ecological implications of hybridization in these species are poorly  
34 studied on a broad geographic scale. Analyses were conducted using leaf morphology, genetic  
35 variation of plastid and nuclear loci, and species distribution models for both species and their  
36 putative hybrid introgressants across 19 localities in California and Arizona, USA. These  
37 analyses revealed evidence for morphological and genetic distinction among localities  
38 comprising putative parental species, but a high degree of morpho-genetic intermediacy among  
39 localities with putative hybrids. Comparison of morphological and genetic population structure  
40 among localities revealed evidence for putative local adaptation or widespread phenotypic  
41 plasticity. Multiple regression models identified a weak but statistically significant negative  
42 association between leaf area and precipitation. Finally, species distribution modeling inferred  
43 northward range shifts over time, with both species predicted to occupy more coastal regions in  
44 the future, possibly increasing the frequency of hybridization among them. These findings  
45 underscore the importance of integrative assessment of multiple data sources in the study of  
46 hybridizing species and highlight the *Rhus integrifolia-ovata* complex as a powerful model for  
47 investigating the adaptive implications of hybridization.

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50 **Keywords:** Hybridization, introgression, California, Arizona, species distribution modeling

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## 54 1 | INTRODUCTION

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56 Hybridization is a hallmark of many plant species complexes, often blurring species boundaries  
57 (Stebbins, 1969; Rieseberg and Soltis, 1991; Petit and Excoffier, 2009). The interplay between  
58 divergent selection among parental species in terms of maximizing reproductive success in a  
59 particular niche vs. the frequency and extent of gene flow remains a key issue in evolutionary  
60 biology (Slatkin, 1987; Burke et al., 1998; Rieseberg et al., 1999; Saccheri and Hanski, 2006;  
61 Sork et al., 2016). The potential outcomes of hybrid introgression are diverse and context-  
62 dependent, including: 1) lower fitness among hybrid offspring reinforcing species boundaries  
63 among distinct parental species (e.g. Rieseberg et al., 1999; Hoskin et al., 2005); 2) higher fitness  
64 among offspring in novel or intermediate environments relative to parental species allowing  
65 invasion of marginal or novel niches, ultimately leading to ecological specialization and eventual  
66 speciation (e.g. Rieseberg et al., 1995; Ellstrand, 2003; Mavarez et al., 2006; Soltis and Soltis,  
67 2009; Abbott et al., 2013); 3) little to no fitness consequences among offspring, with  
68 introgression simply serving as a vehicle for ‘neutral’ genetic exchange among parental species  
69 (e.g. Gavrillets and Cruzan, 1998); 4) the exchange of novel, adaptive genetic variation via gene  
70 flow between parental species through introgressants (Ellstrand and Schierenbeck, 2000; Arnold,  
71 2004; Hegarty and Hiscock, 2004).

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73 A ‘classic’ example of hybrid introgression is that of *R. integrifolia* (Nutt.) Benth. & Hook. f. ex  
74 Rothr. and *Rhus ovata* S. Watson, two ecologically important shrubs native to southwestern  
75 North America (Barkley, 1937; Young, 1974). Both are major structural components of coastal  
76 scrub and chaparral ecosystems, respectively, and are important in erosion control, as native  
77 ornamental shrubs/trees, and as a source of food and shelter for wildlife. *Rhus integrifolia*  
78 occupies coastal scrub habitats in California (USA), Baja California (Mexico), and outlying  
79 islands. *Rhus ovata* occurs in coastal mountains in the chaparral regions of California and  
80 northwestern Mexico and is also disjunct to interior chaparral habitats of central Arizona (USA),  
81 separated from Californian conspecifics by the Sonoran and Mojave deserts (Montalvo et al.,  
82 2017). Both species are gynodioecious, with hermaphroditic and male-sterile individuals  
83 frequently occurring in the same populations. This reproductive strategy has evolved numerous  
84 times in plants and is hypothesized to promote outcrossing and thereby reduce the deleterious  
85 effects of inbreeding depression (Barkley, 1937; Munz and Keck, 1959; Young, 1972; 1974;  
86 Freeman et al., 1997; Barrett, 2002).

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88 *Rhus integrifolia* has relatively small, flat, often toothed, obovate-obelliptic leaves (3-6 cm in  
89 length). *Rhus ovata* has broad, waxy, ovate-deltoid leaves that fold along the midrib of the  
90 abaxial surface into a characteristic “taco” shape, which is likely an adaptation to hot, arid  
91 summers in mid-lower montane chaparral zones (leaves 4-11 cm in length). These morphologies  
92 may represent extremes on an environmental continuum based on proximity to the Pacific  
93 Ocean, moisture, and temperature fluctuations in a diverse, heterogeneous range from coastal  
94 California and Baja California to interior Arizona (Young, 1974; Montalvo et al., 2017).  
95 Californian populations of these two species show varying degrees of morphological  
96 intermediacy due to introgression at intermediate elevations (Figs. 1; S1), where they are often  
97 sympatric; i.e. in regions where the mountains abruptly meet the coast (Barkley, 1937; Young,  
98 1974). Arizonan populations of *R. ovata*, on the other hand, are allopatrically separated from *R.*

99 *integrifolia* or any putatively introgressant populations of *R. ovata*, and thus may represent a  
100 “pure” form of *R. ovata*.

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102 The two species are estimated to have diverged ca. 3 million years ago (mya) +/- 1.6 mya (Miller  
103 et al., 2001, Yi et al., 2004). Fossils attributed to both species have been found at inland sites in  
104 Nevada, farther north than the current distribution of either species, dating back to the Miocene  
105 and even Pliocene (Young, 1974). Thus, these two species may have undergone several periods  
106 of contracting and expanding distributions, being both allopatric and sympatric over hundreds of  
107 thousands to a few million years, e.g. spanning several of the Pleistocene glaciations.

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109 Young (1972; 1974) conducted meticulous studies of the breeding systems and patterns of  
110 introgressive hybridization in these two species, based on samples from two “pure” localities of  
111 each species and one sympatric locality, demonstrating intermediacy in leaf and floral traits in  
112 the sympatric population. However, Young (1974) conceded that the two “pure” populations of  
113 *R. ovata* displayed some intermediate features akin to *R. integrifolia*, and could not rule out that  
114 these populations may be the result of either ‘ancient’ introgression, or introgression in the  
115 immediate past followed by backcrossing with more ‘pure’ forms of *R. ovata*. Further, Young  
116 (1974) found limited evidence for clinal variation in leaf length and width associated with  
117 latitude within *R. ovata*, with shorter, more narrow leaves in the southernmost population  
118 compared to larger, broader leaves in the northern population, though this finding is based on  
119 comparison of only two populations.

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121 Despite these earlier studies, a quantitative assessment of range-wide variation in morphology,  
122 genetic diversity, and abiotic niche requirements is lacking for these two ecologically important  
123 species. Here we use plastid and nuclear DNA sequences, leaf morphometrics, and species  
124 distribution models to characterize patterns of differentiation and hybrid introgression in the  
125 *Rhus integrifolia-ovata* complex, addressing the following questions: 1) What is the extent of leaf  
126 morphological variation across the geographic ranges of *R. ovata* and *R. integrifolia*, and within  
127 introgressed populations, and how to these relate to environmental variation? 2) Do plastid and  
128 nuclear DNA show distinct patterns of population structuring across the allopatric and sympatric  
129 portions of their ranges, and what is the extent of genetic evidence for introgression?  
130 Specifically, are the disjunct Californian and Arizonan populations genetically distinct or do they  
131 display evidence of current or historical introgression (shared haplotypes) with populations in the  
132 sympatric range? 3) How do the inferred environmental niches of *R. integrifolia* and *R. ovata*  
133 differ in the present, past, and future, and what is their degree of niche overlap?

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## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

Leaf material and voucher specimens were sampled from 19 localities across California and Arizona (Table 1). Localities correspond to putatively “pure” populations of *R. ovata* (n = 12), *R. integrifolia* (n = 4), or of ‘mixed’ populations with signs of morphological intermediacy (*R. integrifolia* × *ovata*; n = 3; Fig. 1). No populations were included from the Channel Islands or Baja California due to logistical challenges of collecting material.

### 2.2 | Morphology

Leaves were collected from the basal-most position along a branch, in order to reduce the effect of intra-individual morphological heterogeneity. We were unable to include floral/fruit characters for every locality and instead focused on leaf characters exclusively, which have been shown to be useful indicators of introgression between *R. integrifolia* and *R. ovata* (Young, 1974). Pressed leaves were scanned at 1200 DPI on a Brother TN630 scanner along with a flat 6cm ruler (Fisher Scientific, Waltham, Massachusetts, USA). The software ImageJ2 (Rueden et al., 2017) was used to measure five continuous characters: lamina length, lamina width at widest point, lamina width at 1/4 distance from apex, lamina width at 1/4 distance from base, and petiole length. We scored two meristic characters: number of secondary veins along the adaxial lamina surface on the left side, and the total number of teeth along the lamina margin. We scored seven discrete characters: teeth (present/absent); acute lamina apex (present/absent); base of lamina (acute/truncate/cordate); lamina folding (folded/flat/wavy); red lamina margin (present/absent); basal lamina lobing (present/absent); and lamina shape (ovate-deltoid/oval-elliptic/obovate-obelliptic).

We conducted Principal Components Analysis (PCA) using a correlation matrix in PAST v.3 (Hammer et al., 2001) on all characters and used the ‘biplot’ function to investigate the relative contributions of each character to each resulting PC axis. We used the scree plot and ‘broken stick’ method in PAST to assess the number of PC axes contributing significantly to total variation. We plotted the density of individual scores along PC1 in R using the violin and jitter plot functions in the R package ‘ggplot2’ (Wickham, 2016). We interpreted scores and density of individuals along PC1 as a proxy of a morphological hybrid index among four a priori groupings of taxa/localities: coastal and interior Californian *Rhus ovata*; coastal Californian *R. integrifolia*; interior Arizonan *R. ovata*, and localities with *R. ovata*, *R. integrifolia*, and their introgressants (*R. integrifolia* × *ovata*). We conducted a two-way nonparametric multivariate analysis of variance with Gower transformation for ‘mixed’ data (NP-MANOVA) to evaluate statistically significant multivariate differences in leaf morphology among groupings in PAST v.3, partitioning among two levels: grouping and locality within grouping.

### 2.3 | Leaf area and environmental variation

We constructed multiple regression models in order to investigate the relationship of leaf area to environmental variation. We analyzed the log<sub>10</sub> of leaf area, quantified using ImageJ2, in association with four composite environmental predictor variables. We did not calculate specific leaf area (i.e. leaf area/leaf dry mass) because we were unable to dry the leaves simultaneously under identical conditions, which could have been a source of bias. Nineteen BIOCLIM

183 environmental variables were downloaded for each sampling locality at 2.5 arc-minute resolution  
184 from <https://www.worldclim.org> using the R package ‘raster’ (Hijmans, 2019). Then, PCA was  
185 conducted on the nineteen BIOCLIM variables using a correlation matrix in PASTv.3.  
186 Temperature and precipitation-related variables were analyzed separately in order to investigate  
187 their effects individually. A broken-stick analysis was conducted as above. The first two PCs for  
188 temperature and precipitation were retained for downstream regression analyses. In addition, a  
189 binary grouping variable was included each for localities containing *R. ovata* (CA), *R. ovata*  
190 (AZ), *R. integrifolia* (CA) and *R. integrifolia* × *ovata* (CA) (n = 4 groups), to account for  
191 variation in leaf area among groups.

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193 We included all combinations of PC1 (temperature), PC2 (temperature), PC1 (precipitation),  
194 PC2 (precipitation), and ‘group’ in the models. Relative importance of each term (environmental  
195 PCs, group, and interaction terms) was assessed via significance in the models. The full model  
196 was specified as:  $\log_{10}$  leaf area ~ PC1<sub>temp</sub> + PC2<sub>temp</sub> + PC1<sub>precip</sub> + PC2<sub>precip</sub> + group + interaction  
197 terms + error. Interaction terms included all pairwise combinations of temperature × precipitation  
198 PCs, and of each PC × group. Loadings scores for each BIOCLIM variable with coefficients  
199 above a threshold of 0.25 were interpreted as the variables most strongly associated with each  
200 PC. We ran eight nested models, dropping the *R. integrifolia* (CA) grouping variable for those  
201 models that included a group effect. All analyses were conducted in R using the ‘lm’ function.  
202 Tables were summarized with ‘jtools’ (Long, 2019) and ‘huxtable’ (Hugh-Jones, 2018) in R,  
203 reporting R<sup>2</sup> and R<sup>2</sup>-adjusted values for each model, as well as regression coefficients, standard  
204 errors, and significance for each predictor and interaction term.

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## 206 **2.4 | Plastid and nuclear ITS variation**

207 Approximately 1 cm<sup>2</sup> of leaf material was removed from the center of each leaf adjacent to the  
208 midrib prior to pressing as not to obscure morphological features of the leaf, and DNA was  
209 extracted following a modified CTAB protocol (Doyle and Doyle, 1987), at 1/5 volume. Total  
210 genomic DNA was subjected to PCR amplification of one nuclear and two plastid regions. We  
211 amplified the nuclear internal transcribed spacer (ITS) with primers ITS1 and ITS4 (White et al.,  
212 1990), the plastid *ndhC-trnV*<sup>UAC</sup> spacer with primers rhus-ndhC-F (5’  
213 AGCAGAAACATAGACGAACCTCTCC 3’) and rhus-trnV-R (5’  
214 GTCTACGGTTCGAGTCCGTATAGC 3’), and the plastid *rpl16-rps3* intergenic spacer with  
215 primers rhus-rpl16-F (5’ GGTTCCATCGTTCCCATGCTTCT 3’) and rhus-rps3-R (5’  
216 TGTAGCCGCAGAATAATAAGACT 3’). These regions were chosen based on a previous  
217 assessment of high-variation plastid markers based on complete plastid genomes of *R.*  
218 *integrifolia* and *R. ovata* (NCBI GenBank accession numbers MT024991-MT024993; Barrett, in  
219 review). Reactions were carried out in 25µl volumes, with 12.5µl Apex PCR Master Mix  
220 (Genesee Scientific, San Diego, California, USA), nine µl pure water, 0.2µM of each primer,  
221 0.5µl 5M Betaine, and 20-100ng template DNA in one µl Tris-EDTA Buffer (pH = 8.0). PCR  
222 conditions for ITS consisted of 95°C for 3 min, 30 cycles of 95°C (30 sec), 55°C (45 sec), and  
223 72°C (90 sec), with a final extension of 72°C for 10 min. Conditions for plastid loci differed only  
224 by the annealing step (60°C for 30 sec). PCR products were visualized on 1% agarose gels and  
225 cleaned with 1.8x volume of AxyPrep FragmentSelect magnetic beads (Corning-Axygen,  
226 Corning, New York, USA), followed by two washes with 80% ethanol. PCR products were  
227 cleaned with Sephadex G-50 fine medium (70g/L; GE Healthcare, Chicago, Illinois, USA),  
228 centrifuged through a 96-well filter plate (Phenix Research, Accident, Maryland, USA),



229 quantified via NanoDrop spectrophotometry (ThermoFisher), and diluted to 30ng/μl. PCR  
230 products were sequenced on both strands using the same primers as for amplification, following  
231 manufacturer protocols (Applied Biosystems BigDye v.3.1 cycle sequencing kit, Life  
232 Technologies, Waltham, Massachusetts, USA) on an ABI 3130XL Genetic Analyzer at the West  
233 Virginia University Genomics Core Facility.

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235 Resulting chromatograms were edited in Geneious R10 (<http://www.geneious.com>) and  
236 consensus sequences were aligned with MAFFT v.7 (Katoch and Standley, 2013) under default  
237 parameters (gap opening penalty = 2.0, offset value = 0.5). For ITS electropherograms, all bi-  
238 allelic, heterozygous sites were manually checked in Geneious and scored using IUPAC  
239 ambiguity codes. Alignments for each locus were conducted with MAFFT (gap opening = 3,  
240 offset = 0.5), adjusted manually at the margins in Geneious, and trimmed to remove ambiguous  
241 calls near the priming sites. SeqPhase (Flot, 2010) and PHASE (Stephens et al., 2001) were used  
242 to determine ITS alleles, with a 90% posterior probability per heterozygous site, using sampled  
243 homozygous sequences as prior information. A variable minisatellite repeat in the *ndhC-trnV*  
244 spacer was coded as a single, multistate character (ATT TTT TT[K] ATT ATT AAT TAT T).  
245 Plastid loci were concatenated and analyzed as a single alignment. Sequences are deposited in  
246 NCBI GenBank (Accession numbers: XXXXXXXX-XXXXXXX), and alignment/morphological  
247 data deposited in Dryad (XXXXXX).

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249 Haplotype networks were constructed in PopART v.1.7 (Leigh and Bryant, 2015), using the  
250 ‘TCS network’ option (Clement et al., 2002) with a 95% connection limit. Locality codes and  
251 GPS information (decimal degrees) were then added to a NEXUS file of the plastid and ITS  
252 alignments to map haplotype frequencies using PopART and edited in Adobe Illustrator v. 24.1.1  
253 (Adobe Inc., 2019).

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255 **2.5 | Population genetics**  
256 Population genetic analyses were carried out in ARELQUIN v.3.5 (Excoffier and Lischer, 2010)  
257 for both plastid and nuclear datasets. Analysis of molecular variance (AMOVA; Excoffier et al.,  
258 1992) was conducted for each dataset and partitioned by locality and grouping (as in Table 1) to  
259 quantify the proportion of variation at different hierarchical levels, and to assess the degree of  
260 population structuring across the range of this complex. Pairwise comparisons of  $\Phi_{ST}$  and their  
261 significance were further conducted among localities in ARLEQUIN. The values  $N_{ST}$  and  $G_{ST}$   
262 were compared in SPADS v.1.0 (Dellicour and Mardulyn, 2014) in order to further test  
263 significance of population genetic structure among localities. Population structure was deemed to  
264 be statistically significant if  $N_{ST}$ , which accounts for nucleotide sequence divergence, was  
265 significantly greater than  $G_{ST}$ , which treats alleles as discrete units.

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267 We conducted an overall comparison of the relative degrees of morphological and genetic  
268 variation among localities using the ‘Pstat’ package in R (da Silva and da Silva, 2018). This  
269 package calculates ‘ $P_{ST}$ ’, an analog of ‘ $Q_{ST}$ ’, which may serve as a proxy for genetically  
270 determined morphological variation over a range of levels of additive genetic variation and  
271 narrow-sense heritability. We used individual scores along Reist-transformed (Reist, 1985)  
272 morphological PCs 1 and 2 as metrics of multivariate morphological variation (see above), and  
273 global estimates of  $\Phi_{ST}$  from plastid and nuclear ITS data, respectively. Without information  
274 from common garden and reciprocal transplant experiments (which would have been infeasible

275 for the current study),  $P_{ST} > \Phi_{ST}$  may indicate localized adaptive evolution via divergent  
276 selection among populations but may also reflect plastic responses to environmental variation  
277 (Brommer, 2011). Given the lack of studies on genetically determined morphological variation in  
278 both species of *Rhus* and their introgressant populations, we used this as an exploratory tool to  
279 distinguish a situation in which measures of the degree of morphological variation ( $P_{ST}$ ) outrank  
280 the degree of neutral genetic differentiation ( $\Phi_{ST}$ ) without distinguishing between genetic and  
281 environmental factors, but instead tested this relationship over a range of potential heritability.  
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## 283 **2.6 | Species distribution modeling**

284 Occurrence data were downloaded from the Global Biodiversity Information Facility (GBIF)  
285 using the ‘rgbif’ package for R. The R package ‘CoordinateCleaner’ (Zizka et al., 2018) was  
286 used to filter occurrence data on several criteria, including latitude (below 35°N, corresponding  
287 to the northern limits the native ranges of both species), preserved specimens only, year (post-  
288 1930), occurrences missing coordinates, coordinate uncertainty, and range outliers.  
289

290 We used the R packages ‘raster’ (Hijmans et al., 2019) and ‘sp’ (Pebesma and Bivand, 2005) to  
291 procure 19 BIOCLIM variables from the WorldClim database (Fick and Hijmans, 2017),  
292 corresponding to the cleaned *R. integrifolia* and *R. ovata* datasets based on their GPS coordinates  
293 (10 km resolution), with the addition of the 19 sampled localities from this study. We  $\log_{10}$ -  
294 transformed the BIOCLIM data after adjusting negative and zero values (by adding 100 to these  
295 variables). We subjected the dataset to Principal Components analysis via a correlation matrix in  
296 PAST v.3, and plotted *R. integrifolia* and *R. ovata* occurrence data with the addition of new  
297 collections. We further used NP-MANOVA to test for significant multivariate differences in  
298 environmental variables between *R. integrifolia* and *R. ovata* in PAST.  
299

300 Species distribution models (SDM) were inferred using MaxEnt (version 3.4.1; Phillips et al.,  
301 2006; 2017) via the R package Dismo. Last Glacial Maximum (~22 kya), mid-Holocene (~6  
302 kya), contemporary, and future (IPPC5 2070) bioclimatic predictor variable layers were obtained  
303 via WorldClim (worldclim.org) at 2.5 arc-minute resolution. Last Glacial Maximum and mid-  
304 Holocene predictor layers were generated using the fourth release of the Community Climate  
305 System Model (Gent et al., 2010). We assessed the impact of future climate change on species  
306 distributions by inferring future habitable ranges using both the 2.6 and 8.5 greenhouse gas  
307 representative concentration pathway (RCP) scenarios. Climate layer inclusion was filtered using  
308 Pearson correlation thresholds of 0.85 and -0.85. Analyses of niche equivalency were conducted  
309 per Barrett et al. (2019).  
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## 312 3 | RESULTS

313

### 314 3.1 | Morphology

315 Principal Components Analysis of 14 morphological characters revealed clear separation among  
316 individuals from localities hypothesized to represent “pure” forms of *R. integrifolia* and *R. ovata*  
317 in CA and AZ, as indicated by non-overlapping 95% CIs of PC axes 1 and 2 (Fig. 2A).

318 Individuals from localities with hypothesized introgression between the two species overlapped  
319 broadly in multivariate space with both species. Among localities with *R. ovata*-type  
320 morphologies only, the CA and AZ individuals showed overlap, but the AZ populations only  
321 occupied a relatively small region of multivariate space, with AZ individuals contained within  
322 the 95% CI of the CA individuals. PC1 explained 48.99% of the total variation in morphological  
323 features, while PC2 explained 10.63% (Table 2; S3). PC1 was largely determined by lamina  
324 length/width characters, petiole length, the number of secondary veins, lamina apex shape, leaf  
325 folding pattern, and overall leaf shape (Figs. 2B; S3). PC2 was largely determined leaf shape  
326 characters (overall shape, folding, basal lamina shape), and the pattern of teeth on the lamina  
327 margin.

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329 Morphological variation is further illustrated by a violin plot of PC1 for *R. integrifolia* vs. *R.*  
330 *ovata* (both CA and AZ localities), which showed clear separation among species, with  
331 individuals from introgressant populations covering nearly the entire PC1-ranges of both (Fig.  
332 2C). A two-way NP-MANOVA of Gower-transformed data suggested that the groupings from  
333 Fig. 2A differed significantly in multivariate leaf morphological space (Table S4.,  $F = 9.897$ ,  $p =$   
334  $0.0001$ ), and also that individuals from different sampling localities differed significantly within  
335 each grouping ( $F = 3.511$ ,  $p = 0.0001$ ), with no significant interaction among grouping and  
336 locality factors ( $F = -11.970$ ,  $p = 1.0$ ).

337

### 338 3.2 | Leaf area and environmental variation

339 Principal components analyses of 11 BIOCLIM temperature variables and eight precipitation  
340 variables indicated that the first two temperature and precipitation PCs were significant, based on  
341 a broken stick analysis (Fig. S5). Based on loading scores from the PCA: PC1<sub>temp</sub> reflected  
342 overall temperature magnitude (60.6% of total variance); PC2<sub>temp</sub> reflected temperature variation  
343 (31.1% of total variance, e.g. isothermality, mean diurnal range, annual temperature range);  
344 PC1<sub>precip</sub> reflected overall precipitation magnitude (62.7% of total variance); and PC2<sub>temp</sub>  
345 reflected variation in precipitation (24.3% of total variance, e.g. precipitation seasonality) (Fig.  
346 S5).

347

348 Multiple regression models for  $\log_{10}$  leaf area and environmental variables are summarized in  
349 Table 3. Model 1 included only the four environmental PCs and indicated a significant negative  
350 relationship between PC1<sub>precip</sub> and leaf area (coefficient = -0.04, standard error = 0.01,  $p < 0.01$ ;  
351 reported hereafter as ‘coef’, ‘se, and ‘p,’ respectively). Model 2 included only the binary  
352 grouping variables and indicated that the ‘groups’ each captured a significant amount of  
353 variation in leaf area (coef = 0.20, se = 0.03; coef = 0.30, se = 0.04; coef = 0.33, se = 0.05;  $p <$   
354  $0.001$  for all). Model 3 included environmental PCs and group, further indicating that groups  
355 captured most of the variation in leaf area. Model 4 included environmental PCs and all pairwise  
356 interaction terms between temperature and precipitation PCs, and indicated a significant negative  
357 association between leaf area and PC1<sub>temp</sub> (coef = -0.10, se = 0.02,  $p < 0.001$ ); a significant

358 positive association with PC2<sub>temp</sub> (coef = 0.18, se = 0.04, p < 0.001), and a significant negative  
359 relationship with PC1<sub>precip</sub>. (coef = -0.06; se = 0.02, p < 0.01). This model also indicated a  
360 significant interaction among PC1<sub>precip</sub> and both temperature PCs (coef = -0.03, se = 0.01, p <  
361 0.001 for PC1<sub>temp</sub>; and coef = 0.02, se = 0.00, p < 0.001 for PC2<sub>temp</sub>, respectively).

362  
363 Model 5 included all environmental variables, group, and interactions among environmental  
364 variables. In this model, two of the groups, *R. integrifolia* × *ovata* and Californian *R. ovata*,  
365 showed a significantly positive relationship with leaf area (coef = 0.13, se = 0.04, p < 0.01; coef  
366 = 0.22, se = 0.07, p < 0.01, respectively), while the same two interactions were significant as in  
367 Model 4. Model 6 included all environmental PCs, group, and all group × temperature PC  
368 interactions. Here, PC1<sub>precip</sub> had a significant negative association with leaf area (coef = -0.08; se  
369 = 0.03, p < 0.05), while no other terms were significant. Model 7 was identical to model 6 but  
370 instead included all group × precipitation PC interactions; none of the terms were significant, as  
371 was the case with Model 8 (the ‘full’ model), which included all interactions among group ×  
372 environmental PCs. Thus, there was no further explanatory power by including all interaction  
373 terms.

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### 376 3.3 | Population genetics

377 Fig. 3 reveals a total of 42 phased nuclear ITS haplotypes and 13 plastid haplotypes (combined  
378 *ndhC-trnV* and *rpl16-rps3*). Immediately evident from Fig. 3 is that several ITS haplotypes are  
379 shared among individuals containing *R. integrifolia*, *R. ovata*, and localities with putatively  
380 introgressed individuals. A single ITS haplotype is shared by most individuals of *R. integrifolia*,  
381 with several closely related haplotypes (Fig. 3; 4). A similar pattern of sharing was observed for  
382 plastid DNA (Figs. 3; 4), with greater evidence of structuring among localities than for ITS. Five  
383 plastid DNA haplotypes are present among individuals sampled from *R. integrifolia* localities,  
384 while ten are present among individuals from *R. ovata* from California. A single plastid  
385 haplotype is shared by all AZ individuals of *R. ovata*, while six haplotypes spanning most of the  
386 network are shared among individuals from localities containing *R. integrifolia* × *ovata*.

387 Overall, ITS and plastid haplotype richness is highest for *R. integrifolia* × *ovata* localities (Table  
388 4), followed by *R. ovata* (CA), *R. integrifolia*, and *R. ovata* (AZ), respectively. Nucleotide  
389 diversity is generally the highest in Californian *R. ovata* ( $\pi_{\text{plastid}}$  range = 0.000-0.700;  $\pi_{\text{ITS}}$  range =  
390 0.234-0.780) and *R. ovata* × *integrifolia* localities ( $\pi_{\text{plastid}}$  range = 0.385-0.660;  $\pi_{\text{ITS}}$  range =  
391 0.576-0.771), and lowest in AZ localities of *R. ovata* ( $\pi_{\text{plastid}}$  = 0.000;  $\pi_{\text{ITS}}$  range = 0.000-0.439).

392 AMOVA revealed significant structure among the four groupings for ITS (Table 5;  $\Phi_{\text{CT}}$  = 0.369,  
393 % variation = 55.837, p < 0.001) and plastid DNA ( $\Phi_{\text{CT}}$  = 0.364, % variation = 31.644, p <  
394 0.001). Both ITS and plastid DNA showed significant structure among localities within  
395 groupings, with plastid DNA having higher levels of structuring among localities than nuclear  
396 ITS (Table 4; for plastid DNA  $\Phi_{\text{SC}}$  = 0.557, % variation = 57.023, p < 0.001; for ITS  $\Phi_{\text{SC}}$  =  
397 0.123, % variation = 7.251, p < 0.001). This pattern is further illustrated by pairwise  $\Phi_{\text{ST}}$   
398 comparisons among localities (Fig. 5). Pairwise  $\Phi_{\text{ST}}$  values are generally significant and  
399 relatively high when comparing *R. integrifolia* with *R. ovata* from CA and AZ localities, but  
400 lower compared to *R. integrifolia* × *ovata* localities. A similar overall pattern is observed for  
401 ITS, but in general pairwise  $\Phi_{\text{ST}}$  values are lower, reflecting the results from AMOVA of weaker

402 overall structure among localities relative to plastid DNA.  $N_{ST}$  and  $G_{ST}$  values were 0.786 and  
403 0.696 for plastid DNA, and 0.431 and 0.377 for ITS, respectively. For both plastid DNA and ITS  
404 the differences between  $N_{ST}$  and  $G_{ST}$  were significant (0.090,  $p < 0.001$ ; 0.054,  $p < 0.001$ ,  
405 respectively).

406  
407  $P_{ST}$ - $\Phi_{ST}$  comparisons reveal differentiation in leaf morphology among localities, using the first  
408 two Principal Components as composite proxies (Fig. 6).  $P_{ST}$  was estimated for a range of values  
409 of  $c/h^2$ , which corresponds to the ratio of additive genetic variation in morphology among  
410 localities ( $c$ ) to that among individuals ( $h^2$ , i.e. narrow-sense heritability). Using the estimated  
411 global  $\Phi_{ST}$  values of 0.441 for ITS and 0.684 for plastid DNA, the thresholds for  $c/h^2$  that  
412 correspond to  $P_{ST} > \Phi_{ST}$  are approximately 0.1/0.4 for PC1, and 0.2/0.5 for PC2 (for plastid DNA  
413 and ITS, respectively).

414

### 415 **3.4 | Species distribution modeling**

416 Filtering of occurrence data with CoordinateCleaner retained 503 of 1,209 occurrence points for  
417 *R. integrifolia* and 852 of 1,523 occurrence points for *R. ovata*. PCA of all 19 environmental  
418 variables revealed that the localities sampled only represent a subset of the total multivariate  
419 environmental niche space represented by a larger collection of herbarium records from GBIF  
420 (Fig. S6). PC1 explains 46.3% of the total variation in environmental variables, and is positively  
421 associated with mean diurnal range, temperature seasonality, and temperature/precipitation in the  
422 driest/warmest quarters; PC1 is negatively associated with precipitation seasonality,  
423 isothermality, and temperature during the coldest month/quarter (Table S7). PC2 is positively  
424 associated precipitation (annual, wettest month/quarter, and coldest quarter), and negatively  
425 associated with temperature during the warmest/wettest quarters. Sampling localities for *R.*  
426 *integrifolia*  $\times$  *ovata* fall at intermediate positions between those for *R. integrifolia* and *R. ovata*  
427 from California, while Arizonan *R. ovata* occupy a more distinct portion of multivariate space  
428 associated with warmer, more seasonal habitats (Fig. S6).

429

430 Pearson analysis detected autocorrelation between many bioclimatic predictor variables. The  
431 following variables passed this autocorrelation filter and were used to infer SDMs: annual mean  
432 temperature (1), mean diurnal range (2), isothermality (3), mean temperature of warmest quarter  
433 (8), mean temperature of driest quarter (9), annual precipitation (12), precipitation of driest  
434 month (14), precipitation seasonality (15), precipitation of warmest quarter (18), and  
435 precipitation of coldest quarter (19). MaxEnt inference of species distribution models for both *R.*  
436 *integrifolia* and *ovata* accurately recapitulated their present ranges and showed that the historical  
437 range of *R. ovata* has been highly influenced by climate change over the past ~22,000 years (Fig.  
438 7). The average training AUC for the ten replicate MaxEnt runs using contemporary climatic  
439 variables was 0.988 and 0.977 for *R. integrifolia* and *R. ovata*, respectively. Standard deviation  
440 for training AUC was less than 0.001 for each replicate.

441

442 During the LGM, areas projected to be most habitable for *R. integrifolia* were nearly restricted to  
443 Baja California (Fig. 7). Contrastingly, most of the habitable range of *R. ovata* during the Last  
444 Glacial Maximum was inferred to exist in a relatively narrow band extending from present day  
445 Arizona south to Durango, Mexico. However, the reconstructed SDM for *R. ovata* under a Mid-

446 Holocene climate reflects a pronounced range shift, with most of the habitable area overlapping  
447 that of *R. integrifolia* in Baja California. *Rhus integrifolia* was inferred to gradually shift  
448 northward over time, from Baja California to the central Californian coast. *Rhus ovata*  
449 populations were inferred to have a decreasing probability of occurrence over time in Arizona,  
450 with increasing probability in coastal habitats. Perhaps most interestingly, the area including  
451 present day Arizonan populations of *R. ovata* was inferred to be habitable since the LGM,  
452 suggesting that these populations may have become isolated from the remainder of the range  
453 sometime between 22 and 8 kya. RTR-MO analyses failed to reject niche equivalency of present  
454 ranges ( $p = 0.49$ ). Our SDM projections suggest that the overlap in habitable range for these two  
455 species is likely to increase through 2070, regardless of RCP scenario, as the range of *R.*  
456 *integrifolia* tracks northward along the California coast, and the projected habitability of interior  
457 regions declines for *R. ovata*.  
458

459

## 460 **4 | DISCUSSION**

461

462 Here we present a broad geographic analysis of two ecologically important, hybridizing species,  
463 *Rhus integrifolia* and *R. ovata*. We present morphological and molecular evidence for  
464 distinctness among the two species, but with clear intermediacy in morphology and haplotype  
465 sharing for several localities where they are sympatric. We found population structure in  
466 morphology that outranks neutral genetic structure, which may be driven by either environmental  
467 plasticity or local adaptation. There is weak evidence for a negative association between overall  
468 precipitation and leaf area, but this association is amplified with increasing overall temperature,  
469 and offset by increasing temperature variation. Lastly, we infer that range shifts over the last  
470 ~20,000 years likely reflect periods of both sympatry and allopatry, and that future climate  
471 conditions will favor increased range overlap among the two species in coastal regions and a  
472 decreased probability of *R. ovata* occurrence in Arizona.  
473

474

### 474 **4.1 | Morphology**

475 There is a clear difference among *R. integrifolia* and *R. ovata* based on extensive sampling of  
476 leaf morphological characters, and localities containing both parental species display  
477 intermediate features based on multivariate analyses of morphology (Fig. 2). This intermediacy  
478 is likely to be indicative of the degree to which the genome of each individual is introgressed, i.e.  
479 how much of the genome is represented by each parental species. Based on our sampling,  
480 introgressant populations occur at mid-elevation localities, between approximately 300-400 m  
481 above sea level (Fig. S1). The observed patterns of morphological intermediacy reflect those in  
482 other systems including hybrid introgression in California (e.g. Dorado et al., 1991; Albert et al.,  
483 1997; Dodd and Afzal-Rafii, 2004).  
484

485

486 It is unclear what, if any, positive or negative fitness consequences there may be for introgressive  
487 hybridization in this species complex. It remains to be tested whether selection against alleles  
488 from the other species may limit the spread of these alleles between parental species in the  
489 allopatric portion of their ranges, or if intermediate hybrid populations serve as a bridge for the  
490 exchange of adaptive variation among the two species (Barton and Hewitt, 1985; Rieseberg and  
491 Burke, 2001). While we found morphological differentiation ( $P_{ST}$ ) to outrank neutral genetic



491 differentiation ( $\Phi_{ST}$ ) above a certain heritability threshold (i.e.  $c^2/h = 0.2$  and  $0.4$  for ITS and  
492 plastid DNA, respectively; Fig. 6), it remains unclear whether this is caused by extensive  
493 phenotypic plasticity or adaptive genetic variation.

494

#### 495 **4.2 | Leaf area and environmental variation**

496 Leaf area has long been recognized as an important functional trait at the inter- and intraspecific  
497 levels (e.g. Osnas et al., 2013; 2018). Young (1974) noted a possible relationship between  
498 latitude and leaf size in *R. ovata*, based on limited sampling of three localities. We have  
499 explicitly tested this pattern in the context of abiotic environmental variation, which represents a  
500 more accurate approach than using latitude, longitude, and elevation as proxies for  
501 environmental variation. Our analysis of 19 sampling localities highlights differences among  
502 groupings (e.g. *R. ovata*, *R. integrifolia*, and introgressant populations) as the primary  
503 determinants of leaf area (as corroborated by Fig. 2), but also suggests an association between  
504 overall precipitation ( $PC1_{precip}$ ) and leaf area (Table 3). Although weak, this association is  
505 negative, with smaller leaves at localities with higher overall precipitation. However, there is  
506 interaction between overall temperature + overall precipitation, suggesting these two factors  
507 together may at least partially influence leaf area. This association is offset by temperature  
508 variation; i.e., warmer, wetter areas tend to harbor populations with smaller leaves, but local  
509 climates with more drastic temperature swings show an opposite trend (Table 3).

510

511 Given that that species in xeric regions tend to have leaves that are small (e.g. Givnish et al.,  
512 1979), succulent, dissected, or even absent (e.g. cacti, euphorbias), it seems counterintuitive that  
513 leaf area would decrease with increasing precipitation in this species complex. In fact, we found  
514 the opposite of what has been observed across many other species, as larger leaves are thought to  
515 shed heat more slowly due to a larger boundary layer in hot, arid environments, thus posing a  
516 risk for overheating and extensive evaporative water loss (Schuepp, 1993). *Rhus ovata* has thick,  
517 waxy leaves that fold adaxially along the midrib during the hottest, driest months, which likely  
518 represents an adaptation to seasonally extreme heat and aridity in chaparral habitats (e.g. Herbert  
519 and Larsen, 1985). However, *R. ovata* experiences temperatures below freezing, especially at  
520 high-elevation inland localities (Boorse et al., 1998; Montalvo, 2017), and thus the relationship  
521 observed between leaf area, precipitation, and temperature may reflect a complex tradeoff  
522 between climatic extremes. Other factors not included in our analysis, such as soil fertility,  
523 grazing pressure, and density-dependence may interact with abiotic climatic factors, and provide  
524 a clearer picture of the determinants of leaf area in *Rhus*. There are some localities at which *R.*  
525 *integrifolia*, *R. ovata*, and their hybrids occur within close proximity, with steep elevational  
526 gradients (e.g. in the Santa Monica Mountains of California). These areas provide the ideal  
527 grounds for ‘natural experiments’ to study the dynamics of leaf area as it relates to putative  
528 adaptations to temperature, precipitation, and hybridization.

529

#### 530 **4.3 | Population genetics**

531 Nuclear ITS and two plastid markers display clear patterns of allele sharing at localities with  
532 hypothesized introgression, reflecting a congruent pattern to that of morphological intermediacy  
533 (Figs. 2-4). However, these shared haplotypes are not restricted to localities in which plants  
534 display intermediate morphologies; indeed, the two most common ITS and plastid haplotypes are  
535 widespread, being shared at localities corresponding to either morphologically distinct *R.*  
536 *integrifolia* or Californian *R. ovata*. Within *R. ovata*, which is disjunct from California to



537 Arizona, four of six Californian localities contain multiple plastid haplotypes, whereas all  
538 Arizonan populations contain a single, identical haplotype. Three of five Arizonan localities  
539 contain a single, common ITS haplotype. This finding suggests a potential bottleneck, or that  
540 smaller effective population sizes in Arizona may be prone to the effects of genetic drift,  
541 resulting in overall lower genetic diversity there. Even so, ITS reveals that some haplotypes are  
542 shared widely across the network, differing somewhat from the pattern based on plastid DNA.  
543 For example, the most common ITS haplotype among *R. integrifolia* localities is also found in  
544 CA and AZ localities of *R. ovata*. Likewise, the most common ITS haplotype in AZ localities is  
545 also found in Californian *R. ovata* localities, and even in *R. integrifolia*.

546  
547 Overall, weaker population structure for ITS than for plastid DNA could be driven by two  
548 factors: larger effective population sizes and unsorted ancestral polymorphism for nuclear DNA  
549 than for organellar DNA (Palumbi and Baker, 1994; McCauley, 1995; Avise, 2000; Hare, 2001;  
550 Palumbi et al., 2001), or greater interpopulation dispersal range for pollen vs. seeds (e.g. Ennos,  
551 1994; Hamilton, 1999; Kartzinel et al., 2013). Both *Rhus* species are predominantly pollinated by  
552 bees (Young, 1972; Moldenke and Neff, 1974), while seeds are dispersed by mammals and birds  
553 (Lloret and Zedler, 1991; Rowe and Blazich, 2008). Our findings are congruent with a  
554 hypothesis in which barriers to pollen dispersal are low among populations of both species and  
555 between them, albeit with decreased fecundity for ‘hybrid’ individuals, as observed in previous  
556 experimental crosses (Young, 1972). Negative fecundity barriers may be overcome if pollen flow  
557 occurs frequently enough over long enough distances. Seed dispersal, on the other hand, may be  
558 limited by successful recruitment (e.g. Dunne and Parker, 1999; Arrieta and Suarez, 2006),  
559 which may be amplified if avian or mammalian vectors travel long distances dispersing seeds at  
560 unfavorable localities with different local environmental conditions. This is especially pertinent  
561 if local conditions (soil, temperature, moisture, fire regime, frost formation) vary enough across  
562 sites such that environmental differences between source and sink sites suppress successful  
563 colonization by immigrant propagules, which may not be able to compete in new localities.  
564 Boorse et al. (1998) conducted the only study documenting putative evidence for local adaptation  
565 in *R. ovata*: plants from a site with lower minimum winter temperatures were significantly less  
566 susceptible to freezing damage than plants from a warmer site in the same region of coastal  
567 California. Furthermore, leaves of seedlings were much more susceptible to freezing than those  
568 from adult plants, possibly representing a barrier to successful establishment by propagules from  
569 nearby warmer habitats. Reciprocal transplants using seeds of *R. integrifolia*, *R. ovata*, and their  
570 hybrids could yield useful data on whether recruitment is limited by local adaptation, and  
571 whether this has implications for population structure as it relates to seed dispersal and  
572 successful establishment.

573  
574 Unsorted ancestral polymorphism would seem unlikely in this case to be the sole explanation for  
575 widely shared ITS haplotypes, given the previously estimated divergence time of ~3.1 mya  
576 between *R. integrifolia* and *R. ovata* (Yi et al., 2004). Widespread pollen flow (both historical  
577 and contemporary), potential environmental barriers to external propagule recruitment in  
578 established populations, and differences in plastid vs. nuclear DNA effective population sizes  
579 may all contribute to the discrepancy in population structure among localities. Genome-scale  
580 data would allow several hypotheses to be tested regarding demographic history, patterns of gene  
581 flow between the two species and their introgressants, and the adaptive value (if any) of  
582 introgression. For example, a comparison of which regions of the genome have experienced

583 higher or lower rates of introgression would be particularly insightful, especially in the context  
584 of putatively adaptive or maladaptive introgression (e.g. Grant and Grant, 1998; Rieseberg and  
585 Burke, 2001). Furthermore, these data would provide the level of resolution needed for the  
586 construction of historical demographic models under ancestral and contemporary gene flow vs.  
587 retained ancestral polymorphism.

588

#### 589 **4.4. | Species distribution modeling**

590 Our species distribution models accurately capture the ranges of both species despite the lack of  
591 significant niche differentiation between them. The lack of niche differences between our  
592 MAXENT distribution models based on the RTR-MO test likely reflects the existence of  
593 hybrid/introgressed populations of *R. integrifolia* and *R. ovata* and their high degree of niche  
594 similarity in coastal regions (Fig. 7). Hybrid populations present a problem for species  
595 distribution models; specimens from GBIF are identified either as *R. integrifolia* or *R. ovata* but  
596 do not include information on hybrid status. A meticulous analysis of morphology, and perhaps  
597 even genetic variation from herbarium specimens might improve resolution of future species  
598 distribution models in the *R. integrifolia-ovata* complex, by allowing populations with evidence  
599 of hybrid introgressants to be treated as a separate category. However, even this approach may  
600 be an oversimplification, because the degree to which each population is introgressed is likely to  
601 vary across regions of overlap among the parental species (Arnold, 1997), and thus the  
602 application of a hybrid index on a continuous scale may be more appropriate (e.g. Cullingham et  
603 al., 2012).

604

605 Based on our models, we infer a northward shift in the distribution of *R. integrifolia*, as well as a  
606 higher concentration of occurrence immediately along Californian coast forecasted for 2070, for  
607 both the best- and worst-case projections of future atmospheric CO<sub>2</sub> levels (Fig. 7). Thus, the  
608 distribution of *R. integrifolia* is likely to be forced by climate change to shift into one of the most  
609 densely populated regions in North America, where human development continues to  
610 compromise coastal habitats. For *R. ovata* we infer a similar northward shift for interior  
611 populations in Arizona, and a gradually decreasing occurrence probability in that region. Our  
612 models also infer an increased occurrence probability along the Californian coast and in northern  
613 Baja California for *R. ovata*. Riordan et al. (2018) used species distribution modeling in several  
614 southern Californian chaparral plant species and predicted that suitable habitat would generally  
615 remain stable in the future for *R. ovata*. However, they also predicted that habitat gains in low-  
616 elevation areas for *R. ovata* will likely coincide with future human development, and that  
617 population fragmentation is predicted to increase, with implications for gene flow and local  
618 adaptation.

619

620 Taken together, our 2070 forecast indicates that both species will be forced into a higher degree  
621 of sympatry, possibly increasing the occurrence of introgression. A worst-case scenario would  
622 include decreasing occurrence probability in the allopatric part of the range for *R. ovata* (i.e.  
623 Arizona), coupled with the prediction that *R. integrifolia* and *R. ovata* will be pushed into coastal  
624 regions. Though it is impossible to predict exactly what will happen in the future, global climate  
625 change may contribute to the erosion of locally adapted variants and possibly species boundaries  
626 by increasing the frequency of hybridization among these two species. This finding highlights  
627 the need for investigation of the importance of local adaptation within each species, and the  
628 adaptive consequences of potentially increased levels of hybridization among them, e.g. via

629 common garden experiments, reciprocal transplants with experimental crosses, and genomic  
630 analysis.

631

#### 632 **4.5 | Taxonomic implications**

633 Hybridization has long presented challenges for species delimitation, especially as it relates to  
634 the Biological Species Concept (e.g. Mallet, 2005). While *R. ovata* and *R. integrifolia* are clearly  
635 distinct in regions of allopatry, the same cannot be said within regions of sympatry. The two  
636 species become nearly indistinguishable in the latter, forming a continuous gradation in  
637 morphology, genetic variation, and niche overlap. The evidence presented here does not warrant  
638 specific taxonomic changes, but it does suggest that hybrid status should be considered when  
639 depositing new collections in herbaria or other specimen databases. Furthermore, popular  
640 instruments for the ‘crowdsourcing’ of species occurrence data could be used to help distinguish  
641 among more “pure” forms of each species and their hybrid introgressants, at least for  
642 contemporary observation records. For example, using the application iNaturalist  
643 (<https://www.inaturalist.org/>), there are 170 records of *R. integrifolia* × *ovata* (most likely an  
644 underestimate of their actual abundance), 5,715 of *R. integrifolia*, and 3,591 of *R. ovata* (last  
645 accessed April 14, 2020). While these types of observations have obvious biases (i.e. they tend to  
646 be clustered near population centers or in public lands; e.g. Dickinson et al., 2012), if properly  
647 verified either visually by an expert or via machine learning (e.g. Priya et al., 2012; Wilf et al.,  
648 2016; Kaur and Kaur, 2019) they may ultimately improve distribution models for hybridizing  
649 species, including *R. integrifolia* and *R. ovata*.

650

651 The acquisition of genome scale variation is now feasible for many researchers and should prove  
652 to be particularly informative in determining patterns of gene flow among these two species  
653 (Taylor and Larsen, 2019). Such data will allow the detection of adaptive variants, and regions of  
654 the genome that experience higher or lower levels of gene flow than regions under neutral  
655 expectations (Whitney et al., 2010). Ultimately this type of genomic information could be used  
656 to infer the relative importance of selection in maintaining species boundaries in the face of  
657 frequent gene flow (Rieseberg and Burke, 2001; Feder et al., 2012; Suarez Gonzalez et al.,  
658 2018).

659

#### 660 **5 | CONCLUSIONS**

661 We investigated morphological, genetic, and environmental variation in two ecologically  
662 important, hybridizing species of *Rhus* in the southwestern USA. Our findings revealed morpho-  
663 genetic distinctness among parental species but intermediacy at localities where hybridization is  
664 hypothesized to occur. We further found morphological differences among plants from different  
665 localities that outrank genetic differentiation, suggesting local adaptation or widespread  
666 phenotypic plasticity, and a weak negative relationship between leaf area and overall  
667 precipitation. Species distribution models predicted range shifts northward and into coastal  
668 habitats for both species, possibly with implications for increased future levels of hybridization.  
669 Our study highlights the importance of sampling broadly and integrating morphological, genetic,  
670 and ecological niche data, further underscoring the challenges associated with species  
671 distribution modeling of hybridizing species. Additional studies using reciprocal transplants of  
672 both parental species and their hybrid introgressants, along with genome-wide surveys of  
673 variation will help elucidate the relative impacts of gene flow and selection on the maintenance  
674 of species boundaries.

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## **CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

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**TABLE 1.** Collection locality information for *R. integrifolia*, *R. ovata*, and *R. integrifolia* × *ovata*. US state codes: CA = California, AZ = Arizona.

| Species present                       | Locality Code | Locality  | County, US state  | Latitude, Longitude    | Elevation (m) |
|---------------------------------------|---------------|---|-------------------|------------------------|---------------|
| <i>R. ovata</i>                       | GIAZ-GL       | Globe, Route 77/60, Tonto National Forest                       | Gila, AZ          | 33.552416, -110.677328 | 1330          |
| <i>R. ovata</i>                       | GIAZ-6S       | Six-shooter Canyon, Tonto National Forest                       | Gila, AZ          | 33.339374, -110.784457 | 1218          |
| <i>R. ovata</i>                       | GIAZ-KC       | Kellner Canyon Road, Tonto National Forest                      | Gila, AZ          | 33.334900, -110.830900 | 1343          |
| <i>R. integrifolia</i>                | LACA-PV       | Palos Verdes Peninsula  | Los Angeles, CA   | 33.743931, -118.411408 | 47            |
| <i>R. ovata</i>                       | LACA-CF       | Chantry Flat, Angeles National Forest                           | Los Angeles, CA   | 34.190420, -118.022213 | 605           |
| <i>R. ovata</i>                       | LACA-LH       | Lake Hughes, Angeles National Forest                            | Los Angeles, CA   | 34.560400, -118.491109 | 551           |
| <i>R. ovata</i>                       | SDCA-BG       | Banner Grade, Route 78  | San Diego, CA     | 33.084277, -116.563568 | 307           |
| <i>R. integrifolia</i> × <i>ovata</i> | SDCA-SC       | Sycamore Canyon Road  | San Diego, CA     | 32.942893, -116.985358 | 289           |
| <i>R. ovata</i>                       | SDCA-PM       | Palomar Mountain, South Grade, Cleveland National Forest        | San Diego, CA     | 33.305918, -116.871002 | 1401          |
| <i>R. integrifolia</i>                | SDCA-EF       | Elfin Forest Road   | San Diego, CA     | 33.086753, -117.147970 | 150           |
| <i>R. ovata</i>                       | SDCA-RG       | Rainbow Glen Road   | San Diego, CA     | 33.415497, -117.174885 | 402           |
| <i>R. ovata</i>                       | SBCA-CS       | Cold Spring Trail, Los Padres National Forest                   | Santa Barbara, CA | 34.458688, -119.648858 | 394           |
| <i>R. ovata</i>                       | SBCA-ST       | Snyder Trail, Los Padres National Forest                        | Santa Barbara, CA | 34.536375, -119.789871 | 520           |
| <i>R. integrifolia</i>                | SBCA-EC       | El Capitan State Beach  | Santa Barbara, CA | 34.461526, -120.012142 | 12            |
| <i>R. integrifolia</i>                | VECA-BB       | Beach to Backcountry Trail, Gaviota State Park                  | Santa Barbara, CA | 34.482077, -120.236825 | 133           |
| <i>R. integrifolia</i> × <i>ovata</i> | SBCA-BG       | Los Padres National Forest, near Santa Barbara Botanical Garden | Santa Barbara, CA | 34.467932, -119.708093 | 227           |
| <i>R. integrifolia</i> × <i>ovata</i> | VECA-LJ       | La Jolla Canyon, Los Padres National Forest                     | Ventura, CA       | 34.092930, -119.039087 | 206           |
| <i>R. ovata</i>                       | YAAZ-PR       | Route 89, Prescott National Forest, near Prescott               | Yavapai, AZ       | 34.423816, -112.553975 | 1680          |
| <i>R. ovata</i>                       | YAAZ-BA       | Route 96, Baghdad, AZ   | Yavapai, AZ       | 34.557514, -113.160571 | 1064          |

**TABLE 2.** Morphological character definitions and PCA loadings (PCs 1-2) based on a correlation matrix in PASTv.3.

|  | <b>Character type</b> | <b>PC 1<br/>(48.99%)</b> | <b>PC 2<br/>(10.63%)</b> |
|--|-----------------------|--------------------------|--------------------------|
| <b>Lamina length</b>   | Continuous            | 0.315                    | 0.316                    |
| <b>Lamina width - widest point</b>                                   | Continuous            | 0.354                    | 0.172                    |
| <b>Lamina width - apical half-way point</b>                          | Continuous            | 0.325                    | 0.243                    |
| <b>Lamina width - basal half-way point</b>                           | Continuous            | 0.354                    | 0.157                    |
| <b>Petiole length</b>  | Continuous            | 0.302                    | -0.174                   |
| <b>Number of secondary veins (left side, adaxial surface)</b>        | Meristic              | 0.266                    | 0.229                    |
| <b>Number of teeth</b>   | Meristic              | 0.064                    | 0.291                    |
| <b>Teeth – present/absent</b>  | Binary                | -0.107                   | 0.613                    |
| <b>Leaf apex (acute/round)</b>                                       | Binary                | 0.247                    | -0.161                   |
| <b>Base of lamina (acute, truncate, cordate)</b>                     | Nominal               | 0.223                    | -0.298                   |
| <b>Folding (folded, flat, wavy)</b>                                  | Nominal               | 0.312                    | -0.271                   |
| <b>Red margin (present/absent)</b>                                   | Binary                | -0.257                   | -0.022                   |
| <b>Basal lobing (present/absent)</b>                                 | Binary                | 0.054                    | -0.036                   |
| <b>Lamina shape (ovate-deltoid/oval-elliptic/obovate-obelliptic)</b> | Nominal               | 0.299                    | -0.232                   |

**TABLE 3.** Multiple regression model summary for leaf area vs. PCs 1 and 2 (temperature) and PCs 1 and 2 (precipitation). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . See text for further explanation of the models. Model 8 is not shown, as none of the terms were significant (including all terms and their interactions).

|  | Model 1         |      | Model 2         |      | Model 3         |      | Model 4          |      | Model 5        |      | Model 6        |      | Model 7       |      |
|--|-----------------|------|-----------------|------|-----------------|------|------------------|------|----------------|------|----------------|------|---------------|------|
|  | Coef.           | SE   | Coef.           | SE   | Coef.           | SE   | Coef.            | SE   | Coef.          | SE   | Coef.          | SE   | Coef.         | SE   |
| (Intercept)                                    | 5.91 ***        | 0.01 | 5.74 ***        | 0.02 | 5.75 ***        | 0.04 | 6.00 ***         | 0.04 | 5.84 ***       | 0.06 | 5.47 ***       | 0.56 | 5.14 ***      | 0.44 |
| PC1.temp                                       | 0.00            | 0.01 |                 |      | -0.01           | 0.01 | <b>-0.10</b> *** | 0.02 | -0.06          | 0.03 | 0.18           | 0.32 | -0.01         | 0.02 |
| PC2.temp                                       | -0.02           | 0.01 |                 |      | -0.00           | 0.02 | <b>0.18</b> ***  | 0.04 | 0.09           | 0.05 | -0.39          | 0.58 | -0.03         | 0.03 |
| PC1.precip                                     | <b>-0.04</b> ** | 0.01 |                 |      | -0.00           | 0.02 | <b>-0.06</b> **  | 0.02 | -0.02          | 0.03 | <b>-0.08</b> * | 0.03 | 0.42          | 0.32 |
| PC2.precip                                     | 0.01            | 0.01 |                 |      | -0.02           | 0.01 | -0.05            | 0.03 | -0.04          | 0.04 | -0.04          | 0.02 | -0.36         | 0.21 |
| group [2] <i>R. integ.</i> × <i>ovata</i>      |                 |      | <b>0.20</b> *** | 0.03 | <b>0.20</b> *** | 0.03 |                  |      | <b>0.13</b> ** | 0.04 | 0.65           | 0.58 | 0.78          | 0.43 |
| group [3] <i>R. ovata</i> (CA)                 |                 |      | <b>0.30</b> *** | 0.04 | <b>0.32</b> *** | 0.06 |                  |      | <b>0.22</b> ** | 0.07 | 0.50           | 0.56 | 0.84          | 0.44 |
| group [4] <i>R. ovata</i> (AZ)                 |                 |      | <b>0.33</b> *** | 0.05 | 0.25            | 0.25 |                  |      | 0.50           | 0.28 | -0.13          | 0.60 | <b>1.43</b> * | 0.65 |
| PC1.temp * PC1.precip                          |                 |      |                 |      |                 |      | <b>-0.03</b> *** | 0.01 | <b>-0.02</b> * | 0.01 |                |      |               |      |
| PC1.temp * PC2.precip                          |                 |      |                 |      |                 |      | -0.01            | 0.01 | -0.01          | 0.01 |                |      |               |      |
| PC2.temp * PC1.precip                          |                 |      |                 |      |                 |      | <b>0.02</b> ***  | 0.00 | <b>0.01</b> ** | 0.01 |                |      |               |      |
| PC2.temp * PC2.precip                          |                 |      |                 |      |                 |      | -0.01            | 0.01 | -0.00          | 0.01 |                |      |               |      |
| PC1.temp * group [2]                           |                 |      |                 |      |                 |      |                  |      |                |      | -0.31          | 0.34 |               |      |
| PC1.temp * group [3]                           |                 |      |                 |      |                 |      |                  |      |                |      | -0.17          | 0.32 |               |      |
| PC1.temp * group [4]                           |                 |      |                 |      |                 |      |                  |      |                |      | -0.23          | 0.32 |               |      |
| PC2.temp * group [2]                           |                 |      |                 |      |                 |      |                  |      |                |      | 0.70           | 0.64 |               |      |
| PC2.temp * group [3]                           |                 |      |                 |      |                 |      |                  |      |                |      | 0.36           | 0.59 |               |      |
| PC2.temp * group [4]                           |                 |      |                 |      |                 |      |                  |      |                |      | 0.38           | 0.59 |               |      |
| PC1.precip * group [2]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | -0.39         | 0.32 |
| PC1.precip * group [3]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | -0.52         | 0.32 |
| PC1.precip * group [4]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | -0.36         | 0.33 |
| PC2.precip * group [2]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | 0.33          | 0.21 |
| PC2.precip * group [3]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | 0.30          | 0.21 |
| PC2.precip * group [4]                         |                 |      |                 |      |                 |      |                  |      |                |      |                |      | 0.42          | 0.22 |
| Model R <sup>2</sup> / R <sup>2</sup> adjusted | 0.113 / 0.095   |      | 0.292 / 0.281   |      | 0.305 / 0.280   |      | 0.290 / 0.261    |      | 0.335 / 0.296  |      | 0.332 / 0.285  |      | 0.358 / 0.314 |      |

**TABLE 4.** Nucleotide and haplotype diversity among sampling localities.  $\pi$ -pt = pairwise nucleotide diversity for plastid DNA; A-pt = the number of plastid haplotypes; n-pt = sample size for plastid DNA;  $\pi$ -ITS = pairwise nucleotide diversity for ITS; A-ITS = the number of ITS haplotypes; n-ITS = sample size for ITS.

| Species present                       | Locality code | $\pi$ -pt | A-pt | n-pt | $\pi$ -ITS | A-ITS | n-ITS |
|---------------------------------------|---------------|-----------|------|------|------------|-------|-------|
| <i>R. integrifolia</i>                | LACA-PV       | 0.000     | 1    | 7    | 0.593      | 5     | 14    |
| <i>R. integrifolia</i>                | SBCA-EC       | 0.467     | 2    | 10   | 0.233      | 2     | 16    |
| <i>R. integrifolia</i>                | SDCA-EF       | 0.286     | 2    | 7    | 0.700      | 5     | 16    |
| <i>R. integrifolia</i>                | VECA-BB       | 0.000     | 1    | 19   | 0.225      | 4     | 34    |
| <i>R. integrifolia</i> × <i>ovata</i> | SBCA-BG       | 0.660     | 4    | 18   | 0.771      | 10    | 48    |
| <i>R. integrifolia</i> × <i>ovata</i> | VECA-LJ       | 0.385     | 2    | 13   | 0.576      | 6     | 40    |
| <i>R. integrifolia</i> × <i>ovata</i> | SDCA-SC       | 0.561     | 3    | 23   | 0.691      | 12    | 58    |
| <i>R. ovata</i>                       | SBCA-CS       | 0.700     | 3    | 5    | 0.234      | 2     | 14    |
| <i>R. ovata</i>                       | SBCA-ST       | 0.650     | 1    | 5    | 0.511      | 3     | 10    |
| <i>R. ovata</i>                       | SDCA-BG       | 0.800     | 2    | 5    | 0.533      | 4     | 10    |
| <i>R. ovata</i>                       | LACA-CF       | 0.500     | 2    | 5    | 0.846      | 7     | 10    |
| <i>R. ovata</i>                       | LACA-LH       | 1.000     | 3    | 3    | 0.857      | 5     | 8     |
| <i>R. ovata</i>                       | YAAZ-PR       | 0.000     | 1    | 5    | 0.000      | 1     | 10    |
| <i>R. ovata</i>                       | YAAZ-BA       | 0.000     | 1    | 5    | 0.000      | 1     | 10    |
| <i>R. ovata</i>                       | GIAZ-GL       | 0.000     | 1    | 7    | 0.384      | 3     | 14    |
| <i>R. ovata</i>                       | GIAZ-6S       | 0.000     | 1    | 6    | 0.439      | 3     | 12    |
| <i>R. ovata</i>                       | GIAZ-KC       | 0.000     | 1    | 5    | 0.000      | 1     | 10    |
| <i>R. ovata</i>                       | SDCA-PM       | 0.000     | 1    | 5    | 0.545      | 2     | 12    |
| <i>R. ovata</i>                       | SDCA-RG       | 0.000     | 1    | 7    | 0.78       | 4     | 14    |

**TABLE 5.** Analysis of Molecular Variance (AMOVA, in Arlequin). ‘df’ = degrees of freedom, ‘SS’ = sum of squares, ‘SD’ = standard deviation, ‘% variation’ = percent of total variation explained, ‘ $\Phi$ ’ refers to the analog of inbreeding coefficients (F-statistics) for haploid data, and ‘p’ = significance of differentiation at each hierarchical level.

| <b>Plastid</b>  | <b>df</b> | <b>SS</b> | <b>SD</b> | <b>%<br/>variation</b> | <b><math>\Phi</math></b> | <b>p</b> |
|---|-----------|-----------|-----------|------------------------|--------------------------|----------|
| <b>Within localities (<math>\Phi_{ST}</math>)</b>                 | 2         | 120.701   | 0.473     | 11.332                 | 0.719                    | < 0.001  |
| <b>Among localities within groupings (<math>\Phi_{SC}</math>)</b> | 16        | 331.470   | 2.382     | 57.023                 | 0.557                    | < 0.001  |
| <b>Among groupings (<math>\Phi_{CT}</math>)</b>                   | 152       | 200.881   | 1.322     | 31.644                 | 0.364                    | < 0.001  |
| <b>Total</b>  | 170       | 653.053   | 4.176     | n/a                    |                          | n/a      |
| <b>ITS</b>  |           |           |           |                        |                          |          |
| <b>Within localities (<math>\Phi_{ST}</math>)</b>                 | 2         | 284.812   | 1.142     | 36.875                 | 0.391                    | < 0.001  |
| <b>Among localities within groupings (<math>\Phi_{SC}</math>)</b> | 16        | 90.333    | 0.225     | 7.251                  | 0.123                    | < 0.001  |
| <b>Among groupings (<math>\Phi_{CT}</math>)</b>                   | 345       | 596.835   | 1.730     | 55.873                 | 0.305                    | < 0.001  |
| <b>Total</b>  | 363       | 972.000   | 3.0       | n/a                    |                          | n/a      |

















