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# Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks

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Quantitative knowledge of xylem physical tolerance limits to dehydration is essential to understanding plant drought tolerance but is lacking in many long-vessel angiosperms. We examine the hypothesis that a fundamental association between sustained xylem water transport and downstream tissue function should select for xylem that avoids embolism in long-vessel trees by quantifying xylem capacity to withstand air entry of western North American oaks (*Quercus* spp.). Optical visualization showed that 50% of embolism occurs at water potentials below −2.7 MPa in all 19 species, and −6.6 MPa in the most resistant species. By mapping the evolution of xylem vulnerability to embolism onto a fossil-dated phylogeny of the western North American oaks, we found large differences between clades (sections) while closely related species within each clade vary little in their capacity to withstand air entry. Phylogenetic conservatism in xylem physical tolerance, together with a significant correlation between species distributions along rainfall gradients and their dehydration tolerance, suggests that closely related species occupy similar climatic niches and that species' geographic ranges may have shifted along aridity gradients in accordance with their physical tolerance. Such trends, coupled with evolutionary associations between capacity to withstand xylem embolism and other hydraulic-related traits, yield wide margins of safety against embolism in oaks from diverse habitats. Evolved responses of the vascular system to aridity support the embolism avoidance hypothesis and reveal the importance of quantifying plant capacity to withstand xylem embolism for understanding function and biogeography of some of the Northern Hemisphere's most ecologically and economically important plants.

xylem embolism | plant hydraulic function | drought tolerance | hydraulic safety margins | embolism avoidance hypothesis

Increased rates of drought-induced tree mortality at global scales (1–3) and associated alteration of the land water cycle (4) underscore the timely importance of understanding dynamic plant vascular function during dehydration in order to improve predictions of the composition, productivity, and resilience of woodland and forest communities under future climate conditions (5). Of importance in this regard is elucidating the capacity of the xylem to withstand hydraulic failure as plants desiccate during drought events. Water lost from the internal sites of evaporation within leaf mesophyll—a necessary consequence of a plant's need to maintain rapid gas exchange in leaves for photosynthesis—places the water column in the internal xylem tissue under tension (6) (i.e., negative pressure). This remarkable biophysical process that passively draws moisture up from the hydrated soil through the xylem (6) accounts for ~61% of global land evapotranspiration and returns ~39% of incident precipitation to the atmosphere (7). However, as soil moisture declines during dry periods plants are increasingly unable to replenish the moisture lost from leaves through evapotranspiration (6), making the xylem water column vulnerable to air entry that causes desiccation (i.e., embolisms) (8–10). By reducing the capacity of the xylem to transport water effectively, embolisms isolate downstream tissues (e.g., leaf mesophyll) from water sources in the soil, rendering these tissues susceptible to dehydration and leading to loss of whole-plant function (11, 12) and ultimately plant mortality (5, 13). If, under future climate change scenarios, increasingly common and severe drought events cause dominant plants to embolize more frequently, the potential consequences for global ecosystem function are enormous, including decreased terrestrial productivity, a reduced terrestrial carbon sink, and preferential partitioning of energy fluxes at the land surface into sensible rather than latent heat flux.

Given the importance of quantifying losses of hydraulically mediated whole-plant function and providing accurate predictions of forest productivity and demography under future drought

## Significance

A fundamental association between sustained water transport and downstream tissue survival should select for xylem that avoids embolism in long-lived woody plants. Previous studies suggest that long-vessel species, such as oaks and vines, are more susceptible to drought-induced loss of function than other species. We show that western North American oaks—even those occurring in wet temperate forest—possess xylem capable of tolerating substantial water stress. Evolutionary relationships between drought tolerance traits combined with plant–climate interactions yield positive hydraulic safety margins in oaks from diverse habitats, demonstrating that these key species are not yet on the verge of hydraulically mediated loss of function. Quantifying physical tolerance limits to desiccation is imperative for predicting ecological consequences of future droughts.

ECOLOGY

scenarios (14), it is imperative to have a robust, quantitative understanding of the physical tolerance limits of species to dehydration. Such limits can be quantified from the relationship between xylem water potential and loss of xylem function (so-called xylem vulnerability curves). Species are typically compared by the xylem water potential value at which 50% loss of hydraulic conductance occurs ($P_{50}$) (15), although other reference points may be used in specific physiological contexts, for example, $P_{12}$, $P_{88}$, or $P_{100}$ (5, 16). Studies conducted over three decades have revealed that the hydraulic systems of terrestrial plants as diverse as diffuse porous angiosperms (17) and mosses (18) can withstand low water potentials (less than −1.5 MPa). In conifers, most species have $P_{50}$ values below (i.e., more negative than) −2 MPa [~97% of 96 species in a meta-analysis (15)] and water potentials below the $P_{50}$ threshold have been quantitatively linked to whole-plant mortality under experimentally imposed conditions of water limitation (19). Interspecific variation in $P_{50}$ among conifer species has also been shown to limit the dry end of species distributions along aridity gradients (19–22), as well as being correlated with several other functional, structural, and anatomical traits (20, 22–25).

However, xylem vulnerability to cavitation does not solely determine drought tolerance in woody trees (5, 23, 26). The probability of reaching the critical water potential threshold and the length of time it takes for this to occur are determined by the interaction of several associated physiological and morphological traits (e.g., leaf habit, stomatal closure, minimum cuticular conductance) (5, 23, 26) in relation to the climate and habitat occupied by the species. Consequently, evolutionary relationships between $P_{50}$ and other traits related to water use may yet unify multiple facets of plant form and function (24–27) and produce distinct drought tolerance strategies (28). Understanding how xylem capacity to withstand embolism is integrated with other plant traits is therefore critical for testing ecological and evolutionary hypotheses concerning the diversity of physiological function found in woody plant species.

In this study, we use the recently developed optical method (29, 30) to examine thresholds of hydraulic failure in long vessel [i.e., maximum vessel length >1 m (31)] species of oaks (*Quercus*, Fagaceae) to evaluate the hypothesis that avoiding xylem embolism is a key component of drought tolerance in one of the Northern Hemisphere's most ecologically and economically important angiosperm genera (32). Much uncertainty surrounds the capacity of plants with long vessels to withstand embolism formation and propagation (31, 33, 34) because of methodological difficulties of studying xylem under tension or potential methodological artifacts related to specific techniques for assessing xylem vulnerability to embolism [reviewed extensively by previous authors (33)]. However, recent advances in quantifying the capacity of xylem to withstand air entry and propagation using noninvasive optical techniques, such as X-ray microcomputed tomography (micro-CT) (35, 36) and the optical vulnerability (OV) method (29–31, 34), offer the potential to resolve critical, longstanding issues in plant ecophysiology (31, 34). Although it is not currently feasible to use synchrotron-based micro-CT for the routine study of xylem embolisms in intact plants, many species can be efficiently sampled using the OV method (29, 31), facilitating studies in comparative physiology (31). We asked three related questions: 1) What is the interspecific range in capacity to withstand xylem embolism in *Quercus* of western North America? 2) Are quantitative thresholds of water transport failure in the xylem associated with species geographic distributions on aridity gradients? 3) Does capacity to withstand xylem embolism form part of a whole-plant physiological strategy (i.e., a combination of mechanistically linked responses and characteristics) that leads to embolism avoidance during periods of water deficit? Since variation in traits may be better understood by comparing closely related species in an explicit

phylogenetic context (37, 38), sample species were selected from four sections of *Quercus*, including three clades of western North American oaks and the phylogenetically isolate species *Quercus sadleriana*. Species relationships, for purposes of analysis, follow a recently published fossil-dated phylogeny of the North American oaks (39).

## Results and Discussion

**Spectrum of Xylem Vulnerability to Embolism.** We used the OV method to capture embolism in both leaves and small distal branches (<0.5 cm in diameter) of our 19 sample *Quercus* species. Stem and leaf xylem vulnerability to embolism were strongly, positively correlated with each other when assessed using phylogenetically independent contrast values ($r^2 = 0.56$, $P < 0.0005$, $n = 18$; *SI Appendix*, Fig. S1A) and raw tip species values ($r^2 = 0.70$, $P < 0.0001$, $n = 19$; *SI Appendix*, Fig. S1B). Across all species leaf xylem tended to be slightly more vulnerable than stem xylem (two-factor ANOVA; $F = 8.96$, $P < 0.005$, $n = 19$; *SI Appendix*, Fig. S1B), although this was not universal for individual species (*SI Appendix*, Table S1). We observed no significant difference (Welch two-sample $t$ test, $t = −0.24$, $P = 0.81$) in the water potential difference between stem and leaf $P_{50}$ (so-called xylem vulnerability segmentation) between evergreen (mean = −0.31 MPa; $n = 14$) and deciduous (mean = −0.24 MPa, $n = 5$) species. Hereafter our results focus on stems, because stem xylem vulnerability to embolism is more commonly reported in the literature than leaf xylem vulnerability. However, results of all the analyses were highly similar for both leaves and stems. Stem xylem vulnerability to embolism varied more than twofold among our sample of 19 closely related, long-lived woody angiosperm tree and shrub species (Fig. 1A). Stem $P_{50}$ differed by 3.81 MPa between the most vulnerable (*Q. sadleriana*; $P_{50} = −2.74 \pm 0.20$ MPa) and least vulnerable (*Quercus pacifica*, $P_{50} = −6.55 \pm 0.28$ MPa) species. Very few individuals (<1% of total sample size) exhibited any embolism at water potentials above −2 MPa. Deciduous species (mean $P_{50} = −3.87 \pm 0.19$ MPa, $n = 5$) tended to be more vulnerable to embolism than evergreen species (mean $P_{50} = −4.74 \pm 0.29$ MPa, $n = 14$; Welch two-sample $t$ test, $t = −2.54$, df = 16.5, $P = 0.021$), although this trend was not significant when considered in a phylogenetic context (phylogenetic ANOVA; $P > 0.025$) (Fig. 1 *A* and *B*).

By mapping the evolution of xylem vulnerability to embolism onto the phylogeny, we found large differences in vulnerability to embolism among the different clades (western North American sections and/or series), while closely related *Quercus* species within each clade vary little in their capacity to withstand air entry (Fig. 1B and *SI Appendix*, Table S1). The range of stem $P_{50}$ values was low within the western North American Lobatae series Agrifoliae (maximum difference between species, <0.5 MPa; $n = 4$), and only slightly greater in the Protobalanus (maximum difference between species, <1.5 MPa; $n = 4$) (*SI Appendix*, Table S1 and Fig. 1B). However, stem xylem vulnerability to embolism differed twofold in the *Quercus* section series Dumosae (maximum difference between species, 3.22 MPa; $n = 10$) (*SI Appendix*, Table S1 and Fig. 1B). Ancestral state reconstructions suggest that ancestors in this lineage were more vulnerable to embolism, and had affinities for habitats with higher precipitation (Fig. 2), while the higher resistance to xylem embolism is a more recently derived trait, associated primarily with evergreen shrub forms (Figs. 1B and 3 and *SI Appendix*, Table S1). Significant phylogenetic signal is detected for stem $P_{50}$ when evaluated across all species (Blomberg's $K = 0.63$; $P < 0.05$), indicating that close relatives tend to be similar in this trait [i.e., phylogenetic conservatism (40, 41)].

**Species' Assortment along Aridity Gradients.** *Quercus* species are distributed along a large aridity gradient across western North America (Fig. 2A). Species occur in habitats ranging from mesic
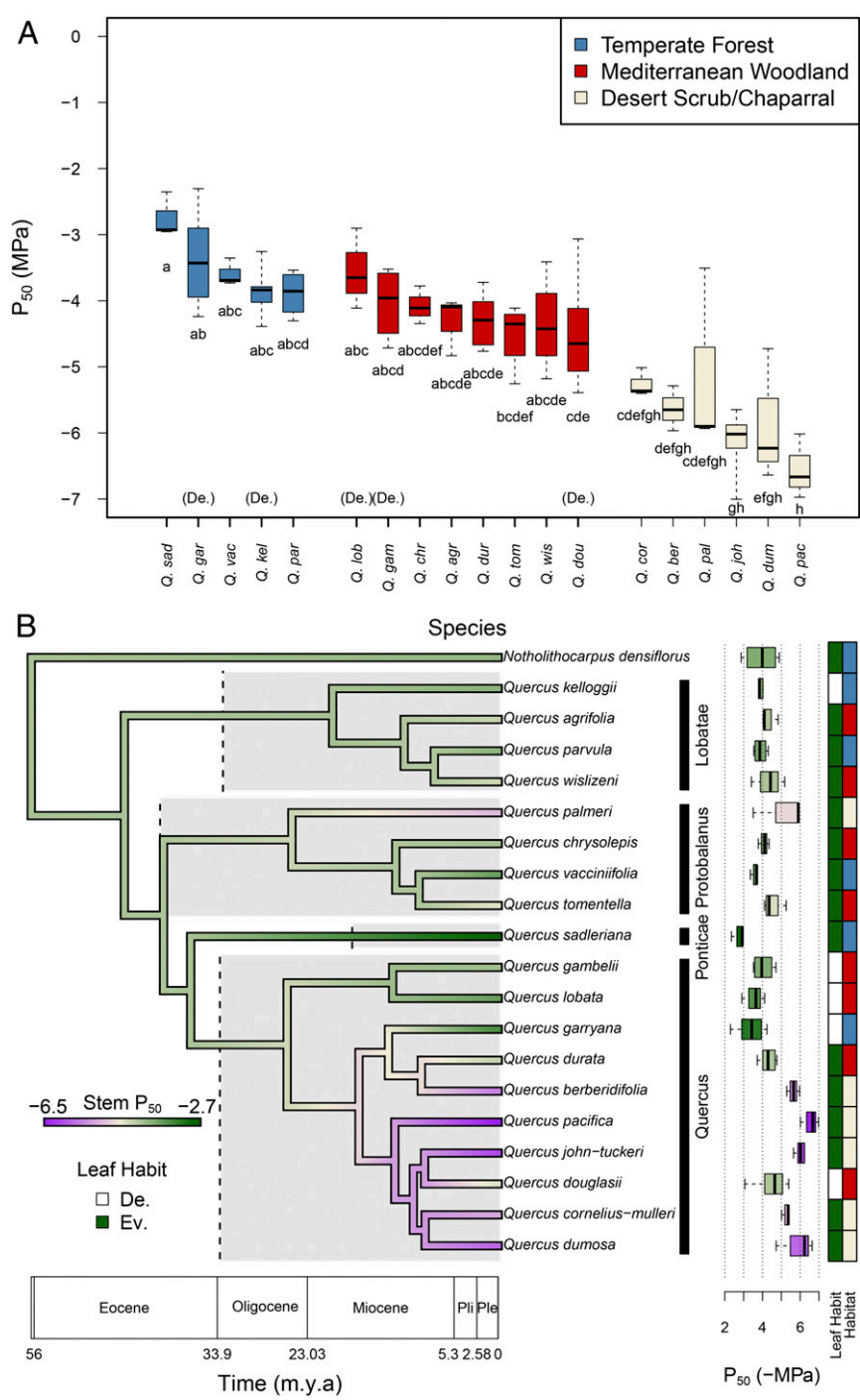
**2 of 9** | PNAS
https://doi.org/10.1073/pnas.2008987118

Skelton et al.
Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks

**Fig. 1.** The spectrum of stem xylem vulnerability to embolism in 19 sample oak species reveals that the xylem can tolerate substantial tensions before substantial levels of embolism are observed. (*A*) The capacity of the xylem to withstand embolism reflects the habitats that species occur in, ranging from mesic temperate forest to xeric desert scrub or chaparral. Boxes indicate interquartile range, thick horizontal black line indicates median, and whiskers indicate full range of values. Letters below each boxplot represent statistically significant ($P < 0.05$) differences between species assessed using ANOVA and post hoc Tukey honest significant difference tests. De., deciduous species. (*B*) Variation in $P_{50}$ across the time-calibrated phylogeny of 19 sample *Quercus* and one sister species from western North America. Sample species are from four different sections of *Quercus* (indicated by black bars). Branch colors reflect changes in stem xylem vulnerability to embolism (stem $P_{50}$) (in megapascals) through time (reconstructed using maximum likelihood). Gray shaded areas indicate western North American clades, with approximate ages of divergence from sister clades indicated by vertical dashed lines. The panel to the *Right* of the phylogeny depicts boxplots of $P_{50}$ for each species. Boxes indicate interquartile range, thick horizontal black line indicates median, and whiskers indicate full range of values. Leaf habit and habitat are indicated. De., deciduous; Ev., evergreen. Colors for the habitat are as in *A*.

temperate rainforest in the Pacific Northwest (e.g., *Q. sadleriana*, *Quercus garryana*) to xeric desert scrub or chaparral in northern Mexico (e.g., *Quercus dumosa*) (Figs. 1*A* and 2*A* and *SI Appendix*, Table S1). Taking advantage of this natural aridity gradient,

we examined the prediction that more arid growing environments will be associated with a greater xylem capacity to withstand air entry. Our results show that species occurring in mesic temperate forest habitats ($n = 6$) tend to have less negative $P_{50}$
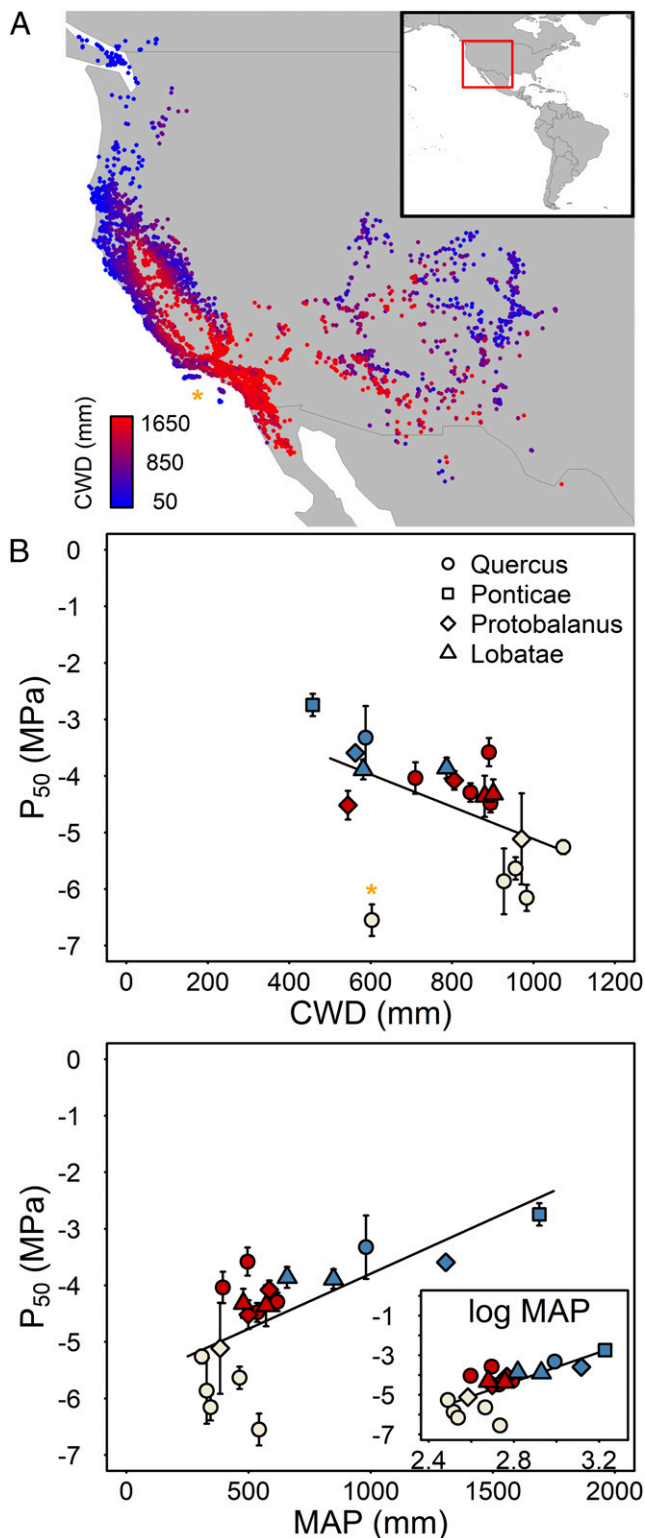
**Fig. 2.** Capacity to withstand xylem embolism in western North American oaks is predictably aligned with climate associated with species' geographic ranges. (*A*) Map of the distribution of *Quercus* species across western North America colored by mean annual climatic water deficit (CWD) (in millimeters) associated with each site. Sites in the Pacific Northwest tend to be much more mesic than sites in the arid south, except for southern offshore island sites (orange asterisk). The *Inset* shows the region in the context of the Americas. (*B*) $P_{50}$ was negatively related to CWD (least-squares regression, $r^2 = 0.26$, $P < 0.05$, $n = 19$) and positively related to mean annual precipitation (MAP) (in millimeters) (least-squares regression, $r^2 = 0.47$, $P < 0.005$,

values than those occurring in either Mediterranean woodlands ($n = 8$), which are in turn less negative than desert scrub/chaparral habitats ($n = 5$) (Fig. 1*A*). Pairwise differences among all three habitat groups are significant (phylogenetic ANOVA; $P = 0.025$ or below). Species from areas with greater mean annual climatic water deficit (i.e., the difference between annual potential evapotranspiration and annual rainfall) tended to have greater xylem capacity to withstand air entry and propagation (Fig. 2*B*). A notable outlier is *Q. pacifica*, the least vulnerable species, which appears to have overbuilt xylem for its moderate growing environment on the Channel Islands (Fig. 2). This outlier in xylem vulnerability could reflect the legacy of historical distributions on the more arid mainland, or the effect of past or infrequent selection events that are not captured in the 20th-century climate records (42). A stronger, positive relationship was observed between mean annual precipitation and $P_{50}$, indicating that species have greater capacity to withstand embolism in lower rainfall environments (Fig. 2*B*). This relationship is significant using both tip values (least-squares regression, $r^2 = 0.47$, $P < 0.005$, $n = 19$) as well as phylogenetic independent contrasts ($r^2 = 0.36$, $P < 0.01$, $n = 18$), and also after log-transforming precipitation to reflect relative instead of absolute differences in rainfall ($r^2 = 0.52$, $P < 0.0005$, $n = 19$). In addition, significant phylogenetic signal is detected for the rainfall associated with species' geographic range (Blomberg's $K = 0.82$, $P = 0.019$), indicating that climate niche is conserved among species. Such a pattern must arise from some combination of ecological processes of traits influencing biogeographical changes (i.e., species' geographic ranges shifting along aridity gradients in accordance with their physiological tolerances) and deeper evolutionary processes of biogeography influencing trait changes (i.e., evolution toward greater capacity to avoid embolism in species inhabiting arid or drying regions) (43). Transitions to lower vulnerability values in species of section *Quercus* series Dumosae likely occurred during the mid to late Miocene, coincident with the emergence of a Mediterranean-type climate in western North America and the onset of drier summers (Fig. 1*B*) (43, 44). While the relative importance of these two processes remains unclear, both imply a key coupling of biogeography with the physiology of drought resistance.

**Whole-Plant Architecture.** We sought to examine whether capacity to withstand xylem embolism is part of a suite of traits related to drought tolerance in *Quercus* by quantifying relationships between $P_{50}$ and plant height and leaf length. A corollary of the embolism avoidance hypothesis is that a high capacity to withstand xylem embolism constitutes a central part of a coordinated drought tolerance strategy in long-vessel, long-lived trees. Because shortened path lengths increase water transport efficiency (45) and smaller leaves decrease evaporative demand (46), we predicted that less vulnerable species would be shorter and have smaller leaves than more vulnerable species. Principal-components analysis (PCA) showed that there is a high degree of coordination of species traits (including maximum plant height, leaf length, and leaf and stem $P_{50}$), where species with more negative $P_{50}$ values tend to be shorter and have smaller leaves than those with higher values (Fig. 3*A*). All four trait variables were correlated with the first principal component (PC1) (loadings >0.44), while height and stem and leaf $P_{50}$ were

$n = 19$) indicating that species' capacity to withstand xylem embolism increases with increasing aridity. The *Inset* shows that $P_{50}$ is positively related to log MAP (least-squares regression, $r^2 = 0.52$, $P < 0.001$, $n = 19$). The orange asterisk indicates *Q. pacifica*, a species endemic to the Channel Islands. Symbols indicate different sections, and colors indicate habitats as shown in Fig. 1 (blue, temperate forest; red, Mediterranean woodland; white, desert scrub/chaparral).
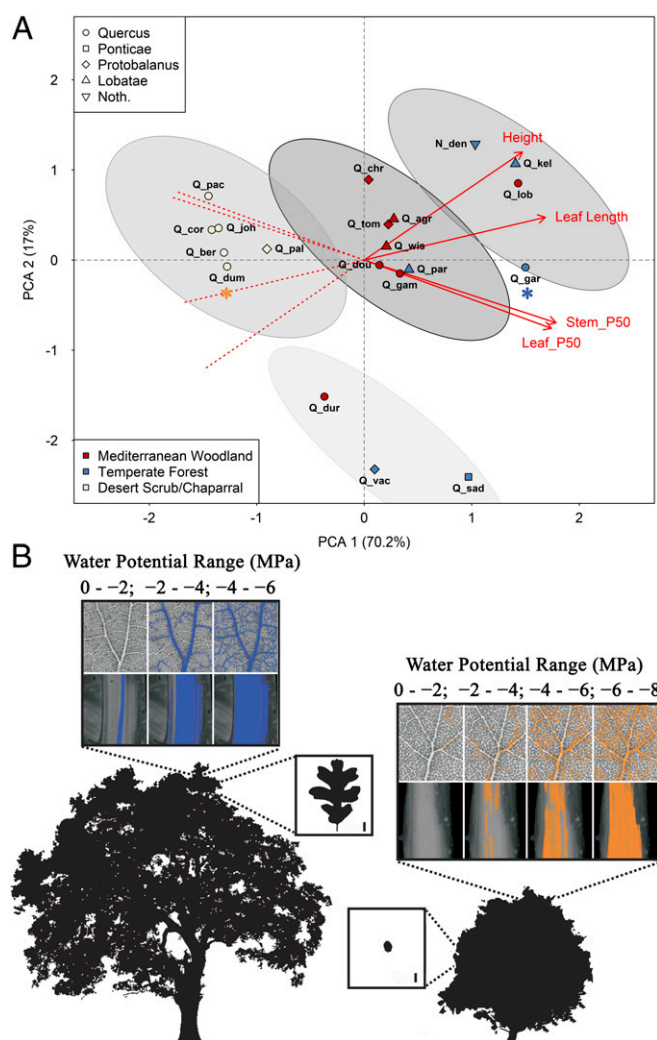
**Fig. 3.** Variation in oak plant height and leaf length reflect changes in stem and leaf xylem vulnerability to embolism resulting in characteristic whole-plant architectures. (*A*) The first two axes of the PCA (accounting for 87.2% of the variance) show the coordination of stem and leaf $P_{50}$, plant height, and leaf length (a proxy for leaf size). Higher values of stem and leaf $P_{50}$ are less negative (and therefore represent xylem that is more vulnerable to air entry than lower values). Species tended to cluster according to habitat type (with few exceptions; see *Results*). Also note the absence of species with large leaves and tall growth forms that have a high capacity to withstand xylem embolism. Plotting symbols indicate different sections; colored by habitat. The blue and orange asterisk indicate species shown in *B* (below). (*B*) Coordination between drought tolerance traits results in characteristic whole-plant architectures in oaks: Species with more vulnerable leaves and stems tend to be tall deciduous trees with larger leaves (e.g., *Q. garryana*; embolism events shown in blue), while more resistant species tend to be shorter evergreen shrubs with entire, small leaves (*Q. dumosa*; embolism events shown in orange). The panel of images depicts xylem embolism events observed over a range of water potentials in leaves (top row) and stems (bottom row). (Scale bar for leaf length: 0.5 cm.)

correlated with PC2. Eigenvalues for axis 1 and axis 2 of the PCA of traits explained 70% and 17%, respectively, of the total variance. The PCA of species traits also showed that species clustered according to habitat (Fig. 3*A*; see also Fig. 1*B*), although there were notable exceptions. *Quercus lobata*, a tall tree from the Mediterranean woodland habitat, was more closely associated with temperate forest species than Mediterranean woodland species in the PCA, while *Quercus parvula* var. *shrevei*, a medium-size tree from the temperate forest habitat, was more

closely associated with Mediterranean woodland species than temperate forest species. Additional water use traits not considered here, such as rooting depth, may allow individuals to experience a microenvironment that matches that of a different habitat. For example, *Q. lobata* may be as tall, as vulnerable, and have leaves as large as species from temperate forest habitats because it reaches supplementary water with deep roots (47). Desert scrub/chaparral species were separated from species occurring in the other two habitats by being shorter, having greater capacity to withstand xylem embolism and smaller leaves. Mediterranean woodland species were intermediate between desert scrub/chaparral and temperate rainforest with respect to these traits. Three species—*Quercus durata*, *Quercus vacciniifolia*, and *Q. sadleriana*—were outliers from the three major species clusters and are shown to have xylem that is vulnerable to embolism, short growth forms, and small leaves. All three of these species occupy uncommon microenvironments within their respective habitats: *Q. durata* is a serpentine endemic; *Q. vacciniifolia* occurs in high elevation sites; and *Q. sadleriana* occurs in very wet understories (and is phylogenetically isolated). Considering phylogeny in the ordination analysis demonstrated that coordinated suites of traits have arisen independently more than once in western North American oaks (Fig. 3*A*). *Quercus palmeri* in section Protobalanus clusters with five species of section *Quercus* series Dumosae, converging on small leaves, short growth form, and greater xylem capacity to withstand embolism. Species from section *Quercus* series Dumosae are present in all three of the main species clusters. Notwithstanding these examples of convergence, significant phylogenetic signal is detected for leaf length ($K = 0.79$, $P < 0.015$) (as well as stem $P_{50}$; see above), but not (log-transformed) height ($K = 0.49$, $P > 0.05$) when evaluated across all species, indicating that close relatives tend to have similar leaf size and xylem vulnerability, but not height.

Pairwise trait comparison of stem $P_{50}$ and height show that species with more negative $P_{50}$ values tend to be shorter than those with higher values using both phylogenetic independent trait values (least-squares regression, $r^2 = 0.32$, $P < 0.01$, $n = 18$) and raw trait values ($r^2 = 0.21$, $P < 0.05$, $n = 19$) (*SI Appendix,* Fig. S2). Quantile regression relationships of the 10th and 90th percentiles produced different slopes. The 90th percentile relationship was significant ($t = 4.4$, $P < 0.0001$), but the 10th percentile relationship was not ($t = 0.3$, $P = 0.77$), suggesting this is a "triangular" relationship, with an absence of tall trees with xylem that is highly resistant to embolism. In addition, stem xylem vulnerability to embolism was positively correlated with maximum leaf length, using both phylogenetically independent contrast values (least-squares regression, $r^2 = 0.47$, $P < 0.001$, $n = 18$) and raw trait values ($r^2 = 0.41$, $P < 0.005$, $n = 19$) (*SI Appendix,* Fig. S2). Like stature, none of the species with highly resistant xylem have large leaves, suggesting the functional incompatibility of this trait combination. Evolutionary associations between $P_{50}$ and other hydraulic-related traits support the view that stem xylem vulnerability to embolism is a critical component of drought tolerance in long-lived woody trees. In *Quercus*, this is reflected in the difference between tall trees with large leaves that are vulnerable to air entry (e.g., *Q. garryana*) versus short shrubs with small, less dissected leaves and high resistance to embolism (e.g., *Q. dumosa*) (Fig. 3*B*).

**Hydraulic Safety Margins.** Finally, we examined whether there is evidence that *Quercus* species avoid major water-stress induced xylem embolism in situ by maintaining positive xylem hydraulic safety margins (i.e., the water potential difference between minimum water potential and $P_{50}$). $P_{50}$ can be used as a proxy for substantial loss of function in plants because it represents the steepest part of the xylem vulnerability response curve. Minimum seasonal water potential is a property integrating climate and whole-plant architecture and/or behaviors (e.g., rooting

Skelton et al.
Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks

PNAS | 5 of 9
https://doi.org/10.1073/pnas.2008987118

depth, rates of leaf water loss). Consequently, if xylem embolism reduces whole-plant functioning (and if plants are architecturally adapted to their environment), hydraulic safety margins should remain positive in all but the most severely dry periods. Our results indicate that most sampled species (over 90%) for which minimum water potential data are available ($n = 14$), including species from all three major habitats in western North America, avoid substantial stem xylem embolism in situ (i.e., have hydraulic safety margins >0 and therefore experience <50% loss of function resulting from water stress; Fig. 4A). The only species with a negative hydraulic safety margin (Q. garryana) was not statistically different from zero (Fig. 4A). Hydraulic safety margins were significantly smaller (Welch two-sample $t$ test; $t = -3.55$, $P < 0.005$) in deciduous species (mean = 0.53 MPa; $n = 5$) than in evergreen species (mean = 1.77 MPa; $n = 9$). A higher risk of leaf embolism under summer drought in deciduous species compared to evergreen species may reflect that these species will replace the leaves in the following year. Although most of our sample Quercus species are winter deciduous, there are confirmed reports of earlier leaf shedding during dry summers, ahead of winter chill (e.g., in Quercus douglasii) (47). Low water potentials have also previously been associated with early onset of leaf abscission (i.e., drought deciduousness) in chaparral shrub species of California (48). Our results also show that individuals of 50% of species avoid water-stress induced xylem embolism in situ (i.e., $P_{min}$ remains less negative than the water potential associated with air entry, $P_{12}$) (Fig. 4A). In 80% of all species the water potential difference between $P_{min}$ and $P_{12}$ was either positive or less than 0.5 MPa (Fig. 4A). Such close coordination of the historical maximum water stress that has been reported for individuals of each species and their evolved xylem tolerance to water deficit is remarkable. Given that previous studies have found limited within species variation in $P_{50}$ (including in one of our study sample species, Q. douglasii (49)), future monitoring of within-species variation in water potential may provide a way of identifying populations that are on the edge of hydraulically mediated loss of function.

Stomatal safety margins (i.e., the water potential difference between turgor loss point [TLP] and $P_{50}$) were positive for all species for which TLP data were available (Fig. 4B; $n = 7$), as reported across diverse short vessel species (17, 26). Furthermore, minimum water potentials for each species were either lower (i.e., more negative) than ($n = 5$) or nonsignificantly different from ($n = 2$) the TLP, indicating that stomata are closed during dry periods. These results suggest that Quercus species (ring-porous species) do not experience embolism with open stomata, consistent with observations in diffuse-porous species (17, 26). Together, our results indicate that stomatal closure is the primary physiological mechanism involved in avoiding water potentials associated with substantial embolism in Quercus, although leaf shedding may also contribute to this process. Large stomatal safety margins also indicate that Quercus species spend substantial time with their stomata closed in dry seasons. Examining whether this introduces carbon limitation in these plants is an important next step in predicting drought impacts in oak-dominated ecosystems.

## Conclusion

Examining xylem vulnerability to embolism of closely related, ecologically diverse Quercus species using the OV method provides insight into fundamental aspects of the functioning, biogeography, and ecology of these long-lived, long-vessel, woody angiosperm tree species. Our results demonstrate that all 19 sampled Quercus species (even those occurring in wet temperate forest) possess xylem capable of withstanding high tension (i.e., water potentials more negative than −2 MPa). Visual techniques have demonstrated similar high capacity to withstand embolism in other long-vessel angiosperm species [e.g., Laurus nobilis (50), Vitis vinifera
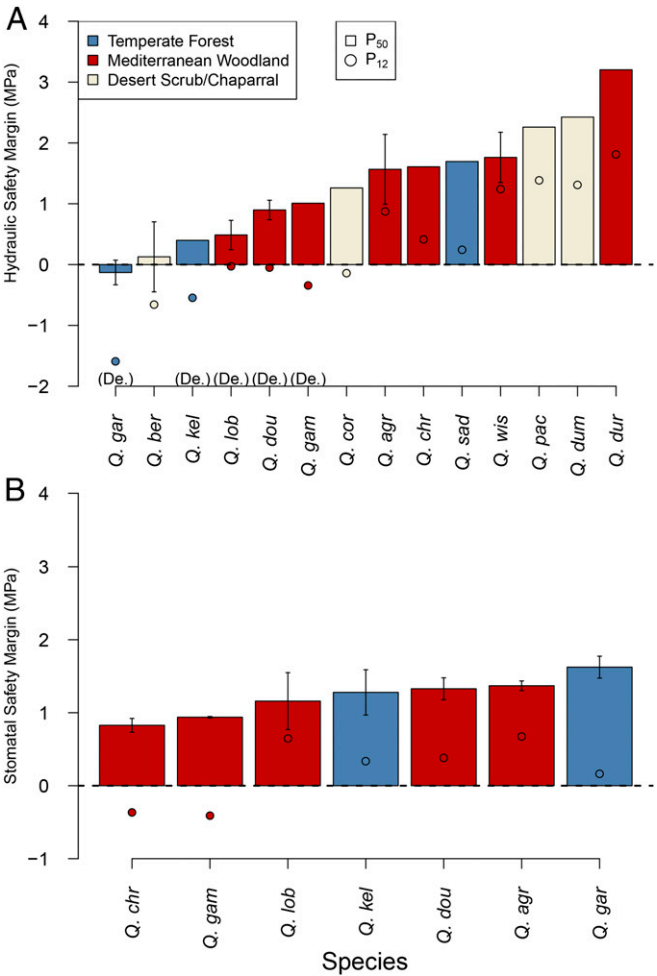


**Fig. 4.** Similar to diffuse porous angiosperms, oaks maintain large hydraulic and stomatal safety margins to avoid loss of hydraulic function. (A) In situ minimum water potentials are either higher than or convergent upon $P_{50}$ values generated using the OV method for a subset of our sample oak species ($n = 14$) from a range of habitats. Positive hydraulic safety margins indicate that species avoid water potentials associated with substantial (>50%) embolism. Error bars represent SE for minimum water potential measurements for each species. Also shown are hydraulic safety margins using $P_{12}$ values instead of $P_{50}$ (circles). De., deciduous. (B) Stomatal safety margins (i.e., the water potential difference between turgor loss point [TLP] and $P_{50}$) were positive for all species for which TLP data were available ($n = 7$). Error bars represent SE for water potential associated with turgor loss for each species. Also shown are stomatal safety margins using $P_{12}$ values instead of $P_{50}$ (circles).

(51), Eucalyptus globulus (30), and Olea europea (52)]. Evolutionary relationships between drought-related traits centering around this capacity to withstand xylem embolism, coupled with plant–climate interactions, are shown to yield positive hydraulic safety margins in Quercus species across a broad range of habitats. These results provide support for the embolism avoidance hypothesis, the central claim of which is that the fundamental association between sustained water transport and downstream tissue survival should select for xylem that avoids embolism in long-lived woody plants (53). Furthermore, coordination among xylem safety, climate, and leaf and plant morphology shown here provides evidence of a unified framework for understanding architecture and drought tolerance in Quercus. Although it remains to be determined whether our findings extend more generally to other long-lived, long-vessel angiosperm species, incorporating these trait relationships into models of oak function is of the utmost importance for predicting hydraulically

mediated function in forests and woodlands dominated by these long-vessel woody tree species.

Our comparative approach shows that xylem vulnerability to embolism of western North American *Quercus* species varies predictably with the aridity associated with their current geographic distributions. At the same time, xylem vulnerability to embolism is also phylogenetically conserved, paralleling within-species studies showing that the trait is highly canalized (54). Habitat filtering combined with trait conservatism suggest that closely related *Quercus* species occupy similar climatic niches in western North America, in contrast with findings from eastern North American oaks where traits that are important for environmental filtering tend to be relatively convergent (55). A continent-wide or global analysis of trait evolution in *Quercus*, using consistent techniques for trait quantification, is needed to understand how trait evolution, regional floristics, and local community assembly processes may contribute to these differences. Our findings also raise the question of whether western North American oaks have the capacity to adapt to rapidly changing climate conditions through natural selection (56). Together, our findings suggest that, although individuals of most of these key species are not yet on the verge of suffering hydraulically mediated loss of distal tissue function, function is likely to be highly impacted by warmer and drier conditions brought upon by rapid climate change.

Accurately determining the physiological and morphological traits of long-vessel angiosperms that confer dehydration resistance will improve predicted responses to drying conditions in biomes where these species currently dominate, including temperate and tropical forests, Mediterranean woodlands, and savannas. The multidimensional nature of trait interactions renders this challenge exceedingly difficult by enabling several possible morphological and functional solutions to tolerating or avoiding water limitation. Seemingly endless possibilities of form and function undoubtedly explain why variation in the vulnerability of plants is often high among coexisting, distantly related species within any single community (57). However, we propose that diversity of plant form among closely related species is driven by a fundamental need to avoid embolism within the xylem that places constraints on other physiological and morphological traits. Revealing the internal mechanisms of plant tolerance to water deficit may greatly assist our ability to identify useful plant functional types for modeling and predicting plant response to future environmental change.

## Materials and Methods

**Sample Collection and Optical Setup.** Large branches of individuals were collected early in the growing season (March to June) in the early morning from 3+ healthy-looking individuals of each species. To avoid any potential artifact associated with open vessels, we ensured that the cut branches were longer than the species' maximum recorded vessel length (maximum branch length, ~2.5 m) (refs. 31 and 58, and references therein). Upon excision, xylem was relaxed by recutting the stems under water. After the cut ends were wrapped in parafilm, branches were immediately placed in at least two plastic bags with damp paper towels to prevent further water loss and transported back to the laboratory at the University of California, Berkeley, for processing. There we used an optical method to capture embolism in both leaves and branches by using flatbed scanners (Epson perfection V800 or V850 Scanner; Epson America) in a dark, temperature-controlled room. Branches from different individuals were used to capture embolism events within the leaves and small branches (<0.5 cm in diameter) according to previously described methods (12, 29–31). We scanned all images at a resolution of 4,200 dpi. Full details, including an overview of the technique, image processing, as well as scripts to guide image capture and analysis, are available at www.opensourceov.org. Also, extensive validation of the techniques can be found in several recent publications (29–31, 34, 36).

**Leaf and Stem Image Collection.** For leaves, we secured a healthy, intact leaf between two microscope slides on a flatbed scanner using duct tape. We scanned each leaf in transmission mode (as opposed to reflective mode, to

allow light to pass through the leaf xylem) at least once every 4 min for a period of a few days (usually <4 d). For stems, we carefully removed a small section of bark from a distal twig (current growing season's growth) to expose the xylem, placed it face down on the scanner, and secured it in place using duct tape. Stems were scanned in reflective mode, which allowed us to observe embolism within the outer few layers of xylem in each stem. The small sizes of the branches that we observed reduced the possibility that our method might have missed significant radial variation in embolism within branches (i.e., between rings), although this possibility cannot be entirely excluded.

**Leaf and Stem Xylem Water Potential.** As branches were being scanned for leaf or stem embolism, we simultaneously monitored stem and leaf xylem water potential. For stem xylem water potential, we attached a stem psychrometer (ICT International) on each branch at more than 60 cm from the cut end of the main branch. Stem psychrometers were connected to the xylem, sealed with high vacuum grease (Dow Corning), and secured with Parafilm (Bemis NA) to prevent moisture loss. Stem xylem water potential was recorded every 10 min for the duration of the scanning process. We verified the accuracy of the stem psychrometer readings for a subset of individuals by periodically measuring leaf xylem water potential using a Scholander-type pressure chamber (PMS Instruments). For leaf xylem water potential, we excised leaves neighboring the scanned leaves, immediately wrapped them in moist paper towel and aluminum foil, and placed them in plastic bags to prevent further water loss. Since branches were largely equilibrated as a result of being kept in the dark, variation among neighboring leaves was slight (always <0.1 MPa).

**Image Processing.** Upon completion, image sequences were analyzed to identify embolism events, seen as changes in the reflection of the stem xylem or changes in the transmission of light through the leaf xylem. Image subtraction of subsequent images conducted in ImageJ (National Institutes of Health, Bethesda, MD) was used to reveal rapid changes in light transmission or contrast produced by each embolism event. Slow movements of the stems or leaves caused by drying could easily be distinguished from embolism events and were filtered from the analysis. Embolism events were thresholded, allowing automated counting of each event using the analyze-stack function in ImageJ. From the thresholded stack of embolism events, we could extract a time-resolved count of embolism events (using the time stamp of each image). We then converted the raw embolism counts to a percentage of total pixels embolized, producing a dataset of time-resolved percent embolism.

**Vulnerability Curves.** The time-resolved percent embolism data were combined with the water potential timeline to estimate the leaf or stem xylem water potential associated with each embolism event. Vulnerability to embolism was recorded as the relationship between percent embolism and water potential ($\Psi$), and modeled using a sigmoid function:

$$\text{Percent embolism} = 100 - 100 \Big/ \left(1 + e^{a(\Psi - b)}\right),$$

where $a$ corresponds to the sensitivity to decreasing water potential (proportional to the slope of the equation) and $b$ is the water potential associated with 50% embolism. In total, our approach yielded 99 stem and 100 leaf vulnerability curves.

*Comparison with previously published vulnerability curves.* We scoured available literature for vulnerability curve data for *Quercus* species, using keywords "xylem vulnerability curves" + "Fagaceae" + "Quercus." Our search found 28 unique records, from which we were able to extract curves for 31 *Quercus* species. These data are shown in *SI Appendix*, Table S4. For the subset of sample species with both OV method and traditional method curves, we compared $P_{50}$ values of our sample group of 19 closely related *Quercus* species to those reported by previous studies but generated using two traditional, nonoptical techniques—the hydraulic method (also referred to as the benchtop dehydration method) and the centrifuge method (*SI Appendix*, Table S4). $P_{50}$ values of our sample group of 19 closely related *Quercus* species are more negative than those reported by previous studies on a subset of the same sample species but generated using traditional, nonoptical techniques (*SI Appendix*, Table S4 and Fig. S3). Furthermore, comparison of all available $P_{50}$ values for *Quercus* species reveals that the centrifuge and hydraulic methods generate significantly higher $P_{50}$ values than the OV method (*SI Appendix*, Fig. S4). Generating different $P_{50}$ values for the same long-vessel species using different techniques could arise due to the following: 1) variation between sample populations arising from local adaptation within species; 2) differences in vulnerability across tissues/xylem ages (59); and/or 3) methodological artifacts associated with particular techniques that produce spuriously low $P_{50}$ values (33). Within-species variation

Skelton et al.
Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks

PNAS | 7 of 9
https://doi.org/10.1073/pnas.2008987118

ECOLOGY

in $P_{50}$ is unlikely to be caused by sampling population, as this does not explain why OV method values are consistently lower than those generated using nonoptical techniques. In addition, several recent studies on conifer or angiosperm tree species [including one on a western North American *Quercus* species (49)] have found limited intraspecific variation in xylem vulnerability to embolism, even between populations from sites across large aridity gradients (49, 60–62). Instead, the most parsimonious explanation for observed differences between methods for *Quercus* species is that the centrifuge and hydraulic methods consistently underestimate xylem vulnerability to embolism of long-vessel species because of open vessel artifacts (33). This conclusion is consistent with claims that other long-vessel angiosperm species (*Vitis vinifera*, *Olea europea*, and *Laurus nobilis*) possess xylem with a higher capacity to withstand embolism than has been previously quantified using centrifuge techniques (50–52).

**Quercus Phylogeny.** We used a time-resolved phylogeny for North American *Quercus* to assess phylogenetic relatedness of our 19 sample species (39). Phylogenetically independent contrasts for all trait data were calculated using the trait correlation function in the *ape* package in R, version 3.6.1. We used Blomberg's *K* to assess the degree of phylogenetic signal (41), comparing the value obtained for each trait to 1,000 values obtained for the same trait values sampled randomly across the phylogeny (63). This was achieved using the *phylosig* function in the *phytools* package in R.

**Species Climatic Niche.** We used species occurrence data from Calflora and Global Biodiversity Information Facility (GBIF) databases to obtain distribution coordinates for sample *Quercus* species. The latter was achieved using the *gbif* function in the *rgbif* package in R. Climate data associated with these sites were extracted from Chelsa, a climate data repository, from which we took the average of all observed values to represent the mean climate value for each species. Climate variables that we assessed were historical (1979 to 2013) mean annual precipitation (MAP) (in millimeters) and climatic water deficit (CWD) (in millimeters) (the difference between mean annual potential evapotranspiration and mean annual precipitation).

**Drought Tolerance Traits.** We used previously published trait data and PCA to examine a prediction of the embolism avoidance hypothesis that a high capacity to withstand xylem embolism constitutes a central part of a coordinated drought tolerance strategy in long-vessel, long-lived trees. Among closely related species, shorter trees have lower total resistance to flow than taller trees by virtue of their shorter pathlengths (45). Larger leaves have greater leaf boundary layer resistance than smaller leaves and impede heat loss more than small leaves do (46). Larger leaves therefore heat up more when exposed to sunlight and tend to have higher leaf temperatures (especially in windless conditions). Because warmer air can hold exponentially more water vapor than colder air, intercellular air spaces in warmer (i.e., larger) leaves hold greater amounts of water vapor at saturation. In addition to requiring more water, this effect also greatly enhances the concentration gradient across the diffusive layer between the leaf mesophyll and the much drier atmosphere, potentially leading to greater water loss in larger leaves. Consequently, we extracted maximum plant height (a proxy for pathlength of the water transport system) and maximum leaf length (as a proxy for leaf size) from previously published sources (64) (data shown in

*SI Appendix*, Table S2) and quantified coordination between these traits and stem and leaf $P_{50}$ using PCA. This was achieved using the *prcomp* function in the *stats* package in R. Furthermore, we used a correlation biplot to indicate the angular relationships among the variables (i.e., to reflect the correlation among the variables) (65, 66).

**Minimum Xylem Water Potential and Turgor Loss Point.** To quantify stomatal and hydraulic safety margins, we extracted turgor loss (TLP) (in megapascals) and minimum xylem water potential ($P_{min}$) (in megapascals) data for our sample *Quercus* species from published literature sources (*SI Appendix*, Tables S2 and S3). We extracted TLP values for 7 out of our 19 sample species (*SI Appendix*, Table S2). For the analysis of hydraulic safety margins, we only included published studies that met the following criteria: 1) xylem water potentials were measured on nonirrigated, field-based, adult plants; 2) mean xylem water potential per site was reported or could be determined for a species, based on at least three individuals; 3) xylem water potential had been quantified toward the end of a dry summer (i.e., between July and October in western North America). This procedure produced $P_{min}$ values for 14 species (*SI Appendix*, Table S3). Where possible, we calculated a mean $P_{min}$ for each species using data from multiple sites and years, to avoid extreme water potential values. Six out of the 14 species had $P_{min}$ values from multiple sites (*SI Appendix*, Table S3). Despite avoiding extreme water potential values, the $P_{min}$ values reported here provide a reasonable proxy for the maximum water stress experienced by individuals of each species because most of them were collected during low rainfall years (*SI Appendix*, Table S3). Quantifying a species mean hydraulic safety margin also assumes that there is limited intraspecific variation in xylem vulnerability to embolism. This assumption was based on several studies showing that there is limited intraspecific variation in xylem vulnerability to embolism (49, 60–62) [including in a western North American *Quercus* species (49)].

**Data Availability.** All study data are included in the article and/or supporting information.

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ECOLOGY

Skelton et al.
Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks

PNAS | 9 of 9
https://doi.org/10.1073/pnas.2008987118