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Evidence for a freezing tolerance–growth rate trade-off in the live oaks (*Quercus* series *Virentes*) across the tropical–temperate divide

Kari Koehler, Alyson Center and Jeannine Cavender-Bares

Department of Ecology, Evolution and Behavior, University of Minnesota, 1987 Upper Buford Circle, Saint Paul, MN 55108, USA

Author for correspondence:
Jeannine Cavender-Bares
Tel: +1 612 624 6337
Email: cavender@umn.edu

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Summary

- It has long been hypothesized that species are limited to the north by minimum temperature and to the south by competition, resulting in a trade-off between freezing tolerance and growth rate. We investigated the extent to which the climatic origins of populations from four live oak species (*Quercus* series *Virentes*) were associated with freezing tolerance and growth rate, and whether species fitted a model of locally adapted populations, each with narrow climatic tolerances, or of broadly adapted populations with wide climatic tolerances.
- Acorns from populations of four species across a tropical–temperate gradient were grown under common tropical and temperate conditions. Growth rate, seed mass, and leaf and stem freezing traits were compared with source minimum temperatures.
- Maximum growth rates under tropical conditions were negatively correlated with freezing tolerance under temperate conditions. The minimum source temperature predicted the freezing tolerance of populations under temperate conditions. The tropical species *Q. oleoides* was differentiated from the three temperate species, and variation among species was greater than among populations.
- The trade-off between freezing tolerance and growth rate supports the range limit hypothesis. Limited variation within species indicates that the distributions of species may be driven more strongly by broad climatic factors than by highly local conditions.

Introduction

A central issue in ecology is to understand the mechanisms that underlie the distributions of species. Climate is known to be a critical factor controlling the broad-scale distribution of organisms (Hutchinson, 1918; MacArthur, 1972; Grace, 1987; Iverson & Prasad, 1998; Rehfeldt *et al.*, 1999, 2006; Cavender-Bares, 2005; Chuine, 2010), but often the physiological basis for climatic niche constraints is unknown. Linking the physiological tolerances of species to their climatic distributions is important in predicting future distributions of species under changing climatic conditions (Morin *et al.*, 2007b). In particular, the freezing tolerance of species often corresponds to the minimum temperatures in their climate of origin (Sakai & Weiser, 1973; Woodward, 1990; Aizen & Woodcock, 1996), and the ability of different species to avoid or tolerate freezing stress through various mechanisms goes a long way to explain the geographic distributions of species (Parker, 1963; George *et al.*, 1974; Latham & Ricklefs, 1993). It has long been hypothesized that the distributions of species are limited by freezing tolerance in the north (in the Northern Hemisphere) and by competition in the south (MacArthur, 1972). A potential mechanism underlying this hypothesis is a trade-off between growth rate and freezing tolerance (Woodward, 1987; Schenk, 1996; Loehle, 1998; Aitken & Hannerz, 2001). In the north, the acquisition of traits for cold acclimation and freezing tolerance has been hypothesized to impose a cost in terms of resource allocation that results in lower growth rates (Levitt, 1980; Beck, 1988; Körner & Larcher, 1988; Woodward, 1990; Howe *et al.*, 2003); a lack of freezing tolerance at the southern edge of the species distribution thus permits higher growth rates and increased competitive ability (Woodward & Pigott, 1975; Woodward, 1987). More generally, increased tolerance to abiotic stress is believed to trade off against growth and competitive abilities in plants (Tilman, 1988) as a result of resource limitations that drive the evolution of allocation strategies. In addition to growth rate, seed mass is considered to be a critical life history attribute associated with season length that may limit southern distributions (Morin & Chuine, 2006). Seed mass contributes directly to absolute growth rates (Cavender-Bares *et al.*, 2004) and is linked to increased survival and competitive ability (Kitajima & Fenner, 2000; Fenner & Thompson, 2005; Turnbull *et al.*, 2008). Towards the tropics, higher seed mass may be favored by a longer season length, which allows a longer time for carbon accumulation, and may provide a

competitive advantage in highly diverse tropical communities in which competitive interactions may dominate assembly processes. By contrast, seed mass may be constrained directly by season length at northern latitudes (Moles & Westoby, 2003; Chuine, 2010), or may be limited by resource investment in freezing tolerance, and large seeds may be less advantageous at northern latitudes where freezing stress is a dominant filter in community assembly.

From a historical biogeographic perspective, if species evolve in tropical regions with no freezing tolerance and then expand into the temperate zone, freezing tolerance may be acquired simultaneously with the evolution of slower growth during unfavorable periods, as both are thought to be advantageous in seasonally cold climates (Larcher & Bauer, 1981; Körner & Larcher, 1988; Kozlowski & Pallardy, 1997). Therefore, a negative relationship between freezing tolerance and growth rate could arise as a result of differential allocation of limited resources or of correlated evolution during range expansion from the tropics to the temperate zone, or both. For similar reasons, a negative relationship between freezing tolerance and seed size may also be expected. Variation in freezing tolerance, as well as both growth rates and seed size, among species could contribute to an explanation of range limits.

Within species, evidence of a decline in growth rate at more northern latitudes and colder temperatures is consistent across studies both *in situ* (Roberds *et al.*, 1990; Matyas & Yeatman, 1992; Rehfeldt *et al.*, 1999, 2001) and in common gardens (Smithberg & Weiser, 1968; Li *et al.*, 1998; Oleksyn *et al.*, 1998). In addition, several studies have shown a similar relationship across species (Rehfeldt, 1997; Yamahira & Conover, 2002; Green, 2007; Savage, 2010). Across species, seed size in forest species has been shown to increase towards the tropics and decrease at high latitudes (Moles & Westoby, 2003; Morin & Chuine, 2006).

A still debated question is whether populations within species show conservatism in their climatic tolerances, a perspective used to defend climatic niche modeling approaches (Wiens & Graham, 2005; Pearman *et al.*, 2008). For example, freezing tolerance may have evolved conservatively, such that all populations within a species (or lineage) have the same resistance to freezing. Under this view, broadly distributed species exposed to a range of temperatures may be adapted to tolerate a wide range of climatic conditions with little local adaptation (Larcher, 2005). Many tree species can survive much colder freezing temperatures than occur in their current range (Fuchigami *et al.*, 1971; Sakai & Weiser, 1973; Aitken & Adams, 1996), suggesting that freezing tolerance may not be costly to maintain in species with broad distributions. Woody species are known to have broad ecological distributions in comparison with herbaceous species, which have more specialized niches (Ricklefs & Latham, 1992). If species have broad and conserved climatic tolerances, little variation in climatic tolerances would be expected among populations.

Within species ranges, however, there is evidence that the distributions of plant species consist of populations genetically suited to local climates (Morgenstern, 1996; Aitken & Hannerz, 2001; Rehfeldt *et al.*, 2001; Larcher, 2005; Cavender-Bares, 2007), and often show clinal variation in the climatic tolerances of species as a result of adaptive differentiation (Endler, 1977; Davis & Shaw, 2001; Rehfeldt *et al.*, 2001; Howe *et al.*, 2003). Clinal variation in freezing tolerance has been demonstrated in several tree species, suggesting high levels of local adaptation (Rehfeldt *et al.*, 2001; Li *et al.*, 2002; Aranda *et al.*, 2005; Morin *et al.*, 2007a; Friedman *et al.*, 2008).

In this study, we examine growth, cold acclimation and freezing tolerance within and between four species in the live oak group (*Quercus* section *Virentes*) – *Q. virginiana*, *Q. oleoides*, *Q. fusiformis* and *Q. geminata* – in a common garden experiment. The live oaks are a small monophyletic lineage containing species in the southern USA and Central America. They are a useful group in which to examine the mechanisms underlying geographic distributions, because they span the tropical–temperate divide, with several species covering large latitudinal gradients. Previous studies have established genetic and morphological differentiation between *Q. geminata* and *Q. virginiana* (Cavender-Bares & Pahlich, 2009) and *Q. virginiana* and *Q. oleoides* (Cavender-Bares, 2007; Cavender-Bares *et al.*, 2011), and there is evidence that the group originated in the tropics of Central America and subsequently expanded into the temperate zone (Cavender-Bares *et al.*, 2011).

Our goals were to establish the extent to which the distributions of live oak species ranges were associated with cold acclimation, freezing tolerance and growth, consistent with the hypothesis that they are limited at the northern range by minimum temperature and at the southern range by competitive ability (growth rate), and whether live oak populations fit a model of local adaptation with narrow climatic tolerances, or of broad climatic tolerances with little variation among populations. We specifically tested, first, whether there was population- and/or species-level variation in the ability to cold acclimate. If more northern populations and/or species increased their freezing tolerance significantly under temperate conditions, but more southern populations did not, this provides evidence for the evolution of cold hardening in response to cold temperature cues. Second, we tested whether there was a trade-off between freezing tolerance and growth rate (both absolute and relative growth rates). A negative relationship, such that maternal lines with slower growth rates exhibit greater freezing tolerance, would provide evidence for this trade-off. We also examined the relationship between freezing tolerance and seed mass, because seed mass can influence competitive ability and has been predicted to vary with climatic distribution (Morin & Chuine, 2006). We note that shade tolerance can drive competitive outcomes in late successional forests (Bazzaz, 1979; Grime, 1979), but we did not examine it here, because all of the live oaks can be characterized as mid-successional savannah species that regenerate under similar (moderately high) light regimes (Kurz & Godfrey, 1962; Spector & Putz, 2006; Klemens *et al.*, 2010). Finally, we tested the extent to which minimum temperatures at the population source predicted freezing tolerance and growth rate. Clinal variation in cold acclimation, freezing tolerance and growth rates among populations within species would provide evidence for local adaptation. Alternatively, large differences in freezing tolerance, growth rates and/or acclimation potential between species, but limited

variation among populations within broadly distributed species, would indicate that species have conserved climatic niches and broad climatic tolerances.

Materials and Methods

Study species and experimental design

The live oaks (*Quercus* series *Virentes* Nixon) consist of seven species of interfertile coastal oaks that span the tropical–temperate divide through the southern USA, Mexico and Central America (Muller, 1961; Nixon, 1985; Nixon & Muller, 1997). Live oaks are evergreen to brevi-deciduous (maintain leaves in mild temperatures, but may drop leaves in response to cold stress) trees characterized by rhizomatous clonal growth, and often occur in shrub form in the juvenile stage in response to low-nutrient soil conditions and seasonal drought. We examined four live oak species (Fig. 1): *Quercus virginiana* Miller (southern live oak), evergreen to brevi-deciduous, occurs in xeric sands to mesic sites from coastal east Texas to Virginia; *Q. geminata* Small, evergreen to brevi-deciduous (sand live oak), occurs in xeric sands to mesic sites from Louisiana to North Carolina; *Q. fusiformis* Small (Texas live oak), evergreen to brevi-deciduous, occurs in xeric sites and open woodlands from Texas to Oklahoma (Nixon & Muller, 1997); and *Q. oleoides* Schltdl. & Cham., a tropical evergreen shrub, occurs in tropical dry forest from the north Pacific coast of Costa Rica to northern Mexico (Boucher, 1983; Nixon, 1985).

Acorns were collected from one to five populations within each of four live oak species: *Q. virginiana* (North Carolina, northern Florida, southern Florida, Louisiana and Texas); *Q. geminata* (northern Florida and North Carolina); *Q. fusiformis* (Texas); and *Q. oleoides* (Mexico, Belize and Costa Rica) (Fig. 1b, Supporting Information Table S1). Coordinates for each tree were recorded (Table S1) and collection permits, export/import permits and phytosanitary certificates were obtained (available on request). Each geographically determined population included five to nine maternal families and *c.* 50 individuals in each family. Seeds were collected from random trees within each population; however, the number of maternal families per population was relatively low and these families may not adequately represent the entire population. Moreover, germination among families was unequal, and some families had low numbers. The length and width of each acorn were measured and the acorns were stored at 4°C until synchronous planting in glasshouse facilities at the University of Minnesota in November 2005.

After 1 yr of growth in a constant temperature and watering regime (tropical conditions), two replicated climate treatments were implemented during the winter months of 2006 (mid-November to March): a tropical treatment, in which daytime temperature was maintained between 30 and 35°C, and night-time temperatures were between 22 and 26°C; and a temperate treatment, in which winter growth temperatures reached a minimum night-time temperature of 4°C with a daytime temperature of 15°C, simulating the monthly average temperatures in Liberia, Costa Rica (southern range limit) and Wilmington, North Carolina (northern range limit). Each climate regime was replicated in two independently controlled glasshouse rooms. The photoperiod was extended to 12 h during the winter, similar to Costa Rica (southern range limit). Summer photoperiods were left at natural levels with a maximum of 15.5 h, which was up to 1 h longer than in North Carolina (northern range limit) and 3 h longer than in Costa Rica. The photoperiod was not a limiting factor in this experiment; however, the decrease from 15.5 to 12 h may have provided some cues for dormancy. These climate treatments were repeated every year for three subsequent years.

Growth rate and seed mass

We compared both the absolute growth rate (AGR) and relative growth rate (RGR) in the growth rate–freezing tolerance trade-off. AGR is the amount of biomass that accumulates in a year and RGR is the maximum intrinsic growth rate. Plants at

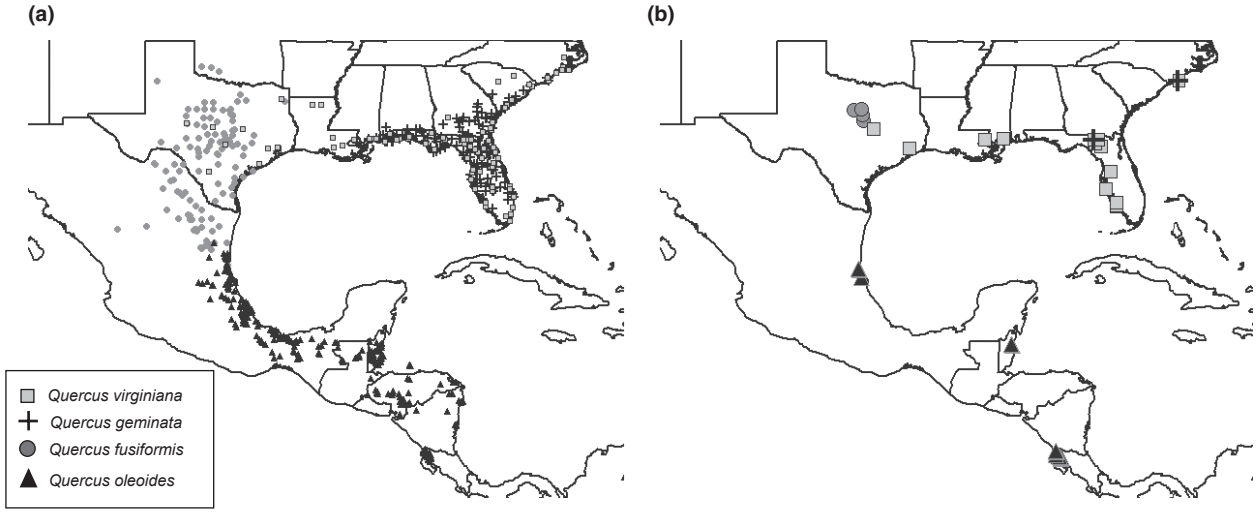

Fig. 1 (a) Range maps for the four live oak species: *Quercus virginiana*, *Q. geminata*, *Q. fusiformis* and *Q. oleoides*, showing herbarium records and collection sites from Cavender-Bares *et al.* (2011). (b) Location of seed sources (large shapes) for each population, using the same symbols as in (a).

northern latitudes may grow more rapidly in early spring, and thus have a higher RGR, but, by the end of the year, may have accumulated less total biomass (AGR) because of a shorter growing season. Therefore, in studies with seasonal variation in growth, the AGR of species under common conditions is the critical factor to compare among populations.

Beginning in early March 2007, 3 months after the start of the winter climate treatment, all individuals were monitored for maximum height (root collar to apex of tallest stem), number of leaves (total leaves on all stems) and stem diameter (measured at the root collar). Allometric equations used to estimate the biomass for each species were developed from data in previous experiments (Cavender-Bares *et al.*, 2004). Above-ground biomass was estimated using the maximum height, total number of leaves and stem diameter. The same equation was used for *Q. virginiana* and *Q. fusiformis*, because of a lack of biomass data for *Q. fusiformis*. Acorn mass was estimated using the acorn volume. AGR (g yr^{-1}) was calculated as AGR = $(M_{final} - M_{initial})/(T_{final} - T_{initial})$, where M_{final} is the biomass estimate from growth measurements made in March 2007, $M_{initial}$ is the estimated acorn mass, T_{final} is the approximate date on which plants were measured in March 2007 and $T_{initial}$ is the date of seedling emergence. RGR (g g^{-1} yr^{-1}) was calculated as RGR = $\log_e(M_{final}) - \log_e(M_{initial})/(T_{final} - T_{initial})$. Seed mass was measured as the seed volume (V) at the time of planting based on the height (h) and diameter (d) of each acorn, assuming an ellipsoid: $V = 4/3 \times (d/2)^2(h/2)$. Fresh seed mass was calculated on the basis of the empirical relationship between volume and fresh mass with the seed coat removed based on seed variation for 480 acorns for 16 species of oak: $M = 0.6363V - 0.1213$ ($R^2 = 0.97$; Cavender-Bares *et al.*, 2004).

Leaf freezing tolerance

In February 2009, freezing manipulations were conducted to determine differences in leaf freezing sensitivity within and between the four species grown in both temperature treatments. Dark-acclimated chlorophyll fluorescence (F_v/F_m) was measured *in situ* to determine the maximum photosynthetic quantum yield for one leaf. Branchlets with at least five leaves, including the measured leaf, were subsequently cut under water, placed in water-filled rose tubes in the dark and frozen in a temperature-controlled freezer box overnight at a minimum temperature of − 10°C. Overnight freezing events were simulated using a custom-designed freezer box (Percival LT-105HID; Percival Scientific, Inc., Perry, Iowa, USA) following previously described methods (Cavender-Bares & Holbrook, 2001; Cavender-Bares *et al.*, 2005; Cavender-Bares, 2007) (Methods S1). To allow recovery, frozen samples were placed in a dark chamber at room temperature (25°C) before the re-measurement of dark-adapted F_v/F_m following Boorse *et al.* (1998). Samples were measured 0.5, 6, 12, 24 and 48 h after the freezing cycle had ended. Approximately nine individuals were measured in each population and climate treatment. The rank order of values for species remained constant across these time points (Fig. S2); for brevity, only values 6 h after freezing are reported further. The decline in F_v/F_m was calculated as F_v/F_m before freezing minus F_v/F_m after freezing. Leaf freezing tolerance was calculated as one minus the decline in F_v/F_m. Leaf cold acclimation ability was calculated as the difference between the tropical and temperate treatment divided by the tropical treatment for decline in F_v/F_m after freezing at − 10°C.

Leaf loss

Leaf loss or the development of a winter deciduous strategy is one adaptation to cold temperatures by which some species avoid freezing damage. Although live oaks often maintain their leaves during the winter months, both field and experimental data indicate that senescence is induced by chilling and varies among populations (Cavender-Bares & Holbrook, 2001; Cavender-Bares, 2007). In March 2009, 4 months after the start of the winter climate treatment, leaf loss was quantified. The percentage of leaf loss for each plant was estimated on the basis of fallen leaves in the pot and leaf scars, and was obtained at the end of the treatment cycle to capture all seasonal loss.

Stem freezing tolerance

In February 2010, electrolyte leakage was measured to determine stem freezing sensitivity. The electrolyte leakage method uses changes in electrical conductivity to predict cell lysis in response to freezing (Flint *et al.*, 1967; Friedman *et al.*, 2008). Stem segments (*c.* 2.2 mm in diameter) were excised from each seedling, the leaves were removed, stems were cut into five 1-cm pieces and each piece was placed in a separate test-tube with 1 ml of deionized water. One tube was stored at 4°C as a nonfrozen control and the other tubes were subsequently frozen at four freezing temperatures (− 5, − 10, − 15 and − 20°C) in a programmable freezing chamber (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) following the methods of Friedman *et al.* (2008) (Methods S1). An average of 20 individuals per population in each climate treatment was measured. The index of injury at each temperature (I_t) was calculated as the percentage of cell lysis at each freezing temperature (Flint *et al.*, 1967). The index of injury data were arcsin transformed for statistical analyses. Stem cold acclimation ability was calculated as the difference between the tropical and temperate treatments divided by the tropical treatment for index of injury after freezing at − 15°C. Stem freezing tolerance was calculated as 100 minus the index of injury at each freezing temperature. Although the rank order of values for stem freezing injury remained relatively constant at the species level at − 10, − 15 and − 20°C, values at − 15°C showed slightly higher differentiation among species (Fig. S2); for brevity, only this measurement is reported further.

Data analysis

Climate data were compiled from WorldClim (Hijmans *et al.*, 2005), using herbarium specimen occurrence localities (GBIF Data Portal, http://www.gbif.org/) to determine climate ranges for all four species. The 19 BioClim climatic parameters and occurrence localities were used in the MAXENT modeling

program to predict species ranges and to determine which climate parameters had the greatest predictive value. The Maxent climate model predicted species distributions very close to actual distributions (Fig. S1); of the 19 BioClim variables, the minimum temperature of the coldest month contributed the most to range predictions for all four species. Therefore, the minimum temperature of the coldest month is used as the climatic variable in all subsequent analyses.

Physiological and growth data for individuals were averaged across maternal families, and maternal families were grouped into populations on the basis of location and species. ANOVAs for all physiological, growth rate and minimum temperature data were conducted with population nested within species to determine species- and population-level differentiation and interactions with climate treatment. Student's *t*-test differentiation was used to determine species and population differences within each treatment. Linear regressions were used to test trade-offs between growth rate, freezing tolerance, acorn mass, cold acclimation and minimum temperature of the coldest month across maternal families. We also used ANCOVA to test whether growth rate and minimum temperature, treated as covariates, predicted stem and leaf freezing tolerance when species, population and climate treatment were included in the model as fixed factors. We obtained restricted maximum likelihood (REML) variance component estimates to determine the percentage of total variation that could be attributed to species and to populations. All analyses were conducted in JMP 7.0 (SAS, Cary, NC, USA).

Results

Climatic distributions

The range of minimum temperatures for the three temperate species (*Q. virginiana*, *Q. geminata* and *Q. fusiformis*) overlapped, but was distinct for *Q. oleoides* (Fig. 2a). The minimum temperatures of the coldest month at the highest probability of presence were as follows: *Q. virginiana* (5°C), *Q. geminata* (8°C), *Q. fusiformis* (7°C) and *Q. oleoides* (16°C). Both *Q. virginiana* and *Q. fusiformis* were distributed across a broad range of minimum temperatures, whereas *Q. geminata* had a narrower climatic range (Fig. 2a).

A hierarchical ANOVA, with population nested within species, showed significant variation in the minimum temperature of the coldest month ($P < 0.0001$; Table 1) between population locations. The northern populations of North Carolina (*Q. virginiana*) and Texas (*Q. fusiformis*) experienced lower minimum temperatures (Fig. 2b). However, Louisiana, Texas and northern Florida populations were not significantly different with regard to the minimum temperature of the coldest month (Fig. 2b). In North Carolina and northern Florida, *Q. geminata* and *Q. virginiana* co-occurred and were exposed to similar minimum temperatures. By contrast, *Q. virginiana* and *Q. fusiformis* in Texas experienced significantly different minimum temperatures (Fig. 2b). *Quercus oleoides* populations occurred in locations with higher minimum temperatures than all populations of *Q. virginiana*, *Q. fusiformis* and *Q. geminata* (Fig. 2b).

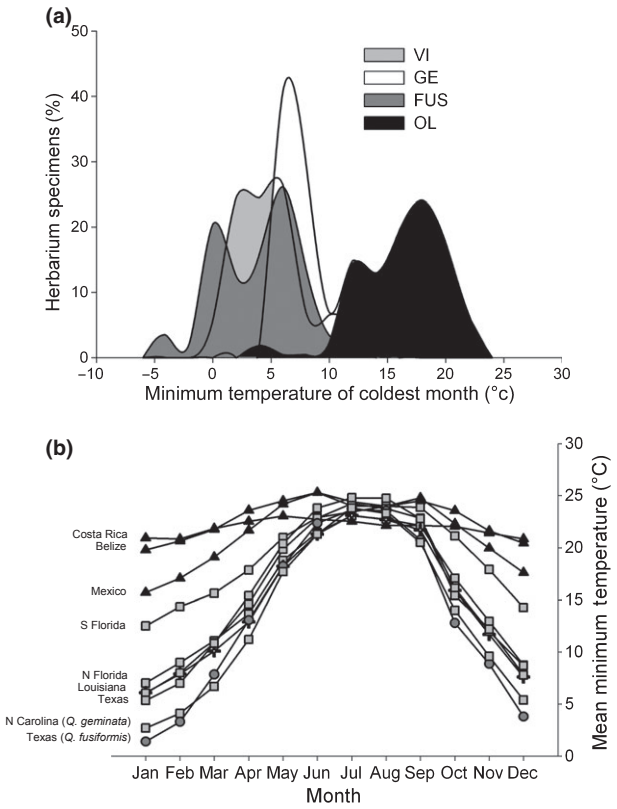

Fig. 2 (a) Climatic distributions for four live oak species based on the percentages of herbarium record occurrences for the minimum temperature in the coldest month (°C). The range of minimum temperatures for the three temperate species (*Quercus virginiana* (VI); *Q. geminata* (GE); *Q. fusiformis* (FUS)) is overlapping, but is distinct for the tropical species *Q. oleoides* (OL). (b) Minimum monthly temperatures for populations within species averaged across maternal family source locations (data from WorldClim; Hijmans *et al.*, 2005): *Q. virginiana*, squares; *Q. geminata*, crosses; *Q. fusiformis*, circles; *Q. oleoides*, triangles. For the North Carolina and northern Florida populations of *Q. virginiana* and *Q. geminate*, the lines are nearly identical and only one is shown. Populations nested within species are significantly differentiated ($P < 0.001$).

Leaf loss

There was almost no leaf loss under tropical conditions for populations or species (Fig. 3a). However, *Q. virginiana* and *Q. fusiformis* exhibited significant leaf loss under temperate conditions ($P < 0.0001$; Table 1; Fig. 3a). Within *Q. virginiana*, Louisiana and Texas populations exhibited greater leaf loss than southern Florida populations (Fig. 3b). Interestingly, *Q. geminata* occurred in the same region as *Q. virginiana* in North Carolina and northern Florida, but did not drop its leaves in response to cold temperatures (Fig. 3b). There was a large amount of variation within *Q. fusiformis*, caused by individuals that either lost very few or almost all of their leaves. Under temperate conditions, 55% of variation was attributed to species-level differences and 2% to population-level differences (Table S2).

Cold acclimation

A hierarchical ANOVA showed significant variation among species for both leaf and stem cold acclimation ability ($P = 0.0002$;

Table 1 ANOVA for leaf and stem freezing tolerance, percent leaf loss, absolute growth rate (AGR), relative growth rate (RGR), minimum temperature of the coldest month from population sources, leaf and stem freezing acclimation

Effect	DF	SS	*F* ratio	*P* value
Leaf freezing tolerance				
Species	**3**	**0.9087**	**31.4613**	**< 0.0001**
Population (Species)	7	0.0997	1.4792	**0.1846**
Growth temperature treatment	**1**	**0.9375**	**97.3764**	**< 0.0001**
Species × treatment	**3**	**0.3426**	**11.8628**	**< 0.0001**
Population (Species) × treatment	**7**	**0.1428**	**2.1189**	**0.0493**
Stem freezing tolerance				
Species	**3**	**8228.4156**	**107.3122**	**< 0.0001**
Population (Species)	**7**	**510.3057**	**2.8522**	**0.0098**
Growth temperature treatment	**1**	**1786.1622**	**69.8835**	**< 0.0001**
Species × treatment	**3**	**613.5215**	**8.0013**	**< 0.0001**
Population (Species) × treatment	*7*	*363.4413*	*2.0314*	*0.0594*
Leaf loss				
Species	**3**	**683.8105**	**18.5006**	**< 0.0001**
Population (Species)	7	127.1160	1.4739	0.1825
Growth temperature treatment	**1**	**376.4443**	**30.5543**	**< 0.0001**
Species × treatment	**3**	**615.3642**	**16.6488**	**< 0.0001**
Population (Species) × treatment	7	103.4442	1.1994	0.3081
AGR (g yr^{-1})				
Species	**3**	**19320.128**	**87.8335**	**< 0.0001**
Population (Species)	**7**	**3307.085**	**6.4435**	**< 0.0001**
Growth temperature treatment	**1**	**1819.123**	**24.8104**	**< 0.0001**
Species × treatment	*3*	*510.702*	*2.3218*	*0.0783*
Population (Species) × treatment	7	587.148	1.144	0.3401
RGR (g g^{-1} yr^{-1})				
Species	**3**	**41.9048**	**47.2661**	**< 0.0001**
Population (Species)	**7**	**22.0294**	**10.6491**	**< 0.0001**
Growth temperature treatment	**1**	**3.6242**	**12.2639**	**0.0006**
Species × treatment	3	0.566	0.6384	0.5916
Population (Species) × treatment	7	0.5873	0.2839	0.9593
Minimum temperature C				
Species	**3**	**6096.1004**	**4131.196**	**< 0.0001**
Population (Species)	**7**	**1142.0569**	**331.6917**	**< 0.0001**
Leaf freezing acclimation				
Species	**3**	**1.9096**	**8.0308**	**0.0002**
Population (Species)	7	1.0257	1.8488	0.1
Stem freezing acclimation				
Species	**3**	**1.69**	**4.5066**	**0.0075**
Population (Species)	7	1.0391	1.1876	0.3286

Significant values ($P < 0.05$) are shown in bold and marginally significant values ($P < 0.1$) are shown in italics. SS, sum of squares.

$P = 0.075$), but populations nested within species were not differentiated significantly (Table 1). For leaf cold acclimation, 35% of the variation was found at the species level and 10% at the population level. For stem cold acclimation, 51% of variation was attributed to species-level differences and 13% to population-level differences (Table S2).

Leaf freezing tolerance

A hierarchical ANOVA showed a significant interaction with climate treatment for both species ($P < 0.0001$) and population nested within species ($P = 0.0493$; Table 1). Plants grown under tropical conditions all showed less leaf freezing tolerance than plants acclimated to cold temperatures, and showed no significant differentiation in response to freezing at either the population or species level (Fig. 3c,d). Cold-acclimated plants showed differentiation in leaf-level freezing tolerance at the species level: the temperate species *Q. geminata*, *Q. virginana* and *Q. fusiformis* all showed greater freezing tolerance than the tropical species *Q. oleoides* (Fig. 3c).

Under temperate conditions, within *Q. virginiana*, the North Carolina population showed greater freezing tolerance than the northern and southern Florida populations, exhibiting some population-level variation (Fig. 3d). However, there were no differences between the northern Florida and North Carolina populations of *Q. geminata* with regard to leaf freezing tolerance. *Quercus oleoides* showed the lowest stem freezing tolerance. Under temperate conditions, 69% of the variation was attributed to species-level differences and 5% to population-level differences (Table S2).

Stem freezing tolerance

A hierarchical ANOVA showed significant interaction with climate treatment for species ($P < 0.0001$), and population nested within species was marginally significant ($P = 0.0594$, Table 1). Cold-acclimated plants showed species-level variation after freezing at − 15°C (Fig. 3e). *Quercus oleoides* showed the lowest freezing tolerance across both climate treatments (Fig. 3e). In the temperate treatment, *Q. geminata* showed less freezing tolerance than *Q. virginiana* or *Q. fusiformis*.

Within *Q. oleoides*, there was limited population-level differences. However, the Mexican population exhibited greater stem freezing tolerance than the Costa Rican or Belizean populations (Fig. 3f), corresponding to a lower minimum temperature in Mexico (Fig. 2b); the same pattern was not seen in leaf freezing tolerance, however (Fig. 3d). Under temperate conditions, within *Q. virginiana*, the North Carolina population showed less stem freezing damage than the northern and southern Florida populations, exhibiting some population-level variation corresponding to latitude (Fig. 3f). However, there were no differences between the northern Florida and North Carolina populations of *Q. geminata*. Under temperate conditions, 82% of the variation in stem freezing tolerance was attributed to species and 5% to population differences (Table S2).

Growth rate

A hierarchical ANOVA showed significant variation between species ($P < 0.0001$) and population nested within species ($P < 0.0001$; Table 1). However, interactions with climate treatment were not significantly different. In general, AGR and RGR showed similar patterns in species-level response (Fig. 3g,i). Growth rates were reduced in response to the temperate treatment

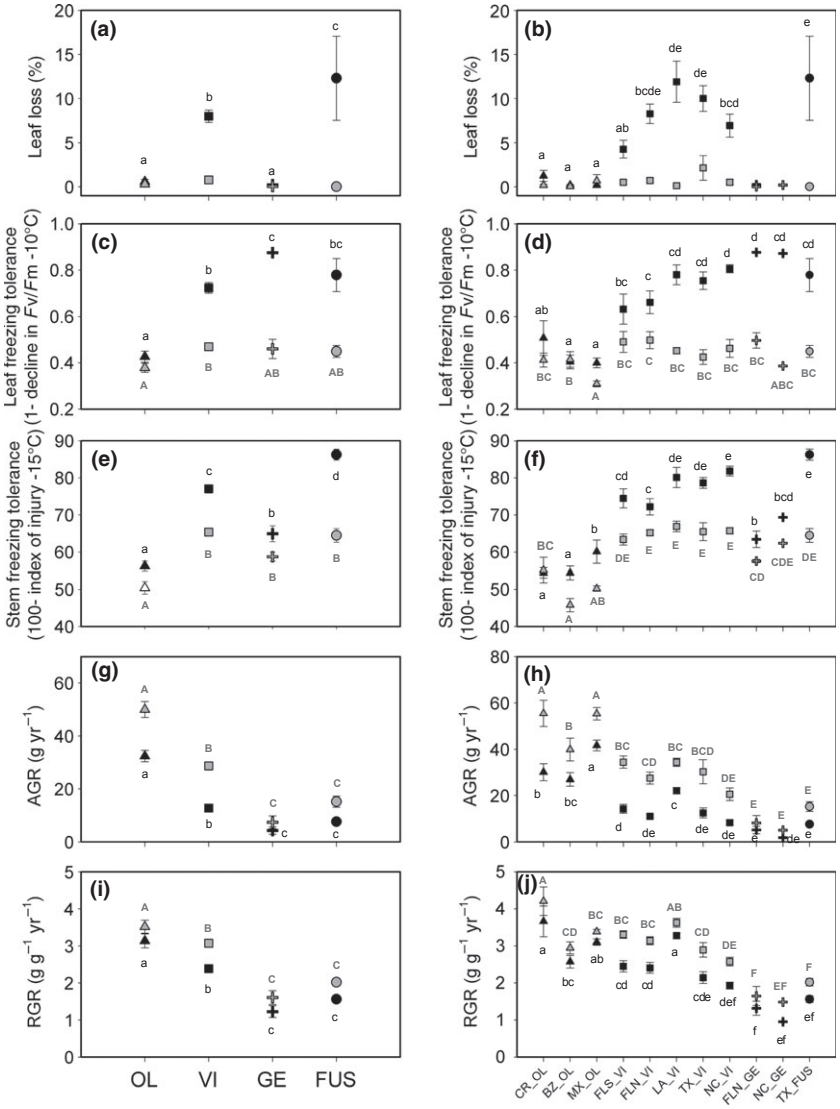

Fig. 3 Species (left column) and population (right column) means (± SE) under controlled tropical (gray) and temperate (black) climatic conditions for leaf loss (a,b), leaf (1 − decline in F_v/F_m after freezing at − 10°C) (c,d) and stem (100 − index of injury after freezing at − 15°C) (e,f) freezing tolerance and absolute (AGR) (g,h) and relative (RGR) (i,j) growth rates. Species: *Quercus virginiana*, VI, squares; *Q. geminata*, GE, crosses; *Q. fusiformis*, FUS, circles; *Q. oleoides*, OL, triangles. Populations: *Quercus oleoides*, CR_OL, Costa Rica; BZ_OL, Belize; MX_OL, Mexico; *Q. virginana*, FLS_VI, southern Florida; FLN_VI, northern Florida; LA_VI, Louisiana; TX_VI, Texas; NC_VI, North Carolina; *Q. geminata*, FLN_GE, northern Florida; NC_GE, North Carolina; *Q. fusiformis*, TX_FUS, Texas. Different letters indicate significant Student's *t*-test species- and population-level differentiation ($P < 0.05$) within the tropical and temperate treatments.

relative to the tropical treatment for all species (Fig. 3g,i). Cold-acclimated plants showed species-level variation (Fig. 3g,i). *Quercus oleoides* showed a reduced AGR in the temperate treatment, but maintained a higher growth rate than any of the temperate species (Fig. 3d). In addition, *Q. fusiformis* and *Q. geminata* had lower growth rates than *Q. virginiana*.

Within *Q. virginiana*, cold-acclimated plants in the Louisiana population had a higher growth rate (AGR and RGR) than other *Q. virginiana* populations, similar to some populations of *Q. oleoides* (Fig. 3h,i). For AGR, 71% of the variation was attributed to species-level differences and 13% to population-level differences. For RGR, 54% of the variation was attributed to species-level differences and 17% to population-level differences (Table S2). Seed mass differed significantly among species, but not among populations within species (Fig. S2). Seed mass was weakly correlated with AGR (R^2 = 0.092, P = 0.009, Fig. 5f), a relationship driven largely by species; relatively little of the variation in AGR among families was explained by seed mass. Seed mass was not correlated with RGR (Fig. S4).

Trade-offs between freezing tolerance, growth and seed mass across the tropical–temperate gradient

We found evidence for a trade-off between growth and freezing tolerance across four live oak species (Fig. 4a–c). The most appropriate test for a trade-off between growth and freezing tolerance is between growth rates under nonstressed (tropical) conditions and freezing tolerance under cold-acclimated (temperate) conditions. This comparison was possible, given that seeds from the same mother tree were planted in both treatments and family mean values for nonstressed growth and cold-acclimated freezing could be compared directly. Families with higher AGRs had lower freezing tolerance in both stems and leaves (Fig. 4a,c). The same was true for RGR and stem freezing tolerance, but there was no relationship between RGR and leaf freezing tolerance. Both leaf and stem freezing tolerance were negatively correlated with seed mass (Fig. 4e,f), although the relationship with stem freezing tolerance was stronger. Cold acclimation ability in both leaves (Fig. 4g) and stems (Fig. 4h) was negatively

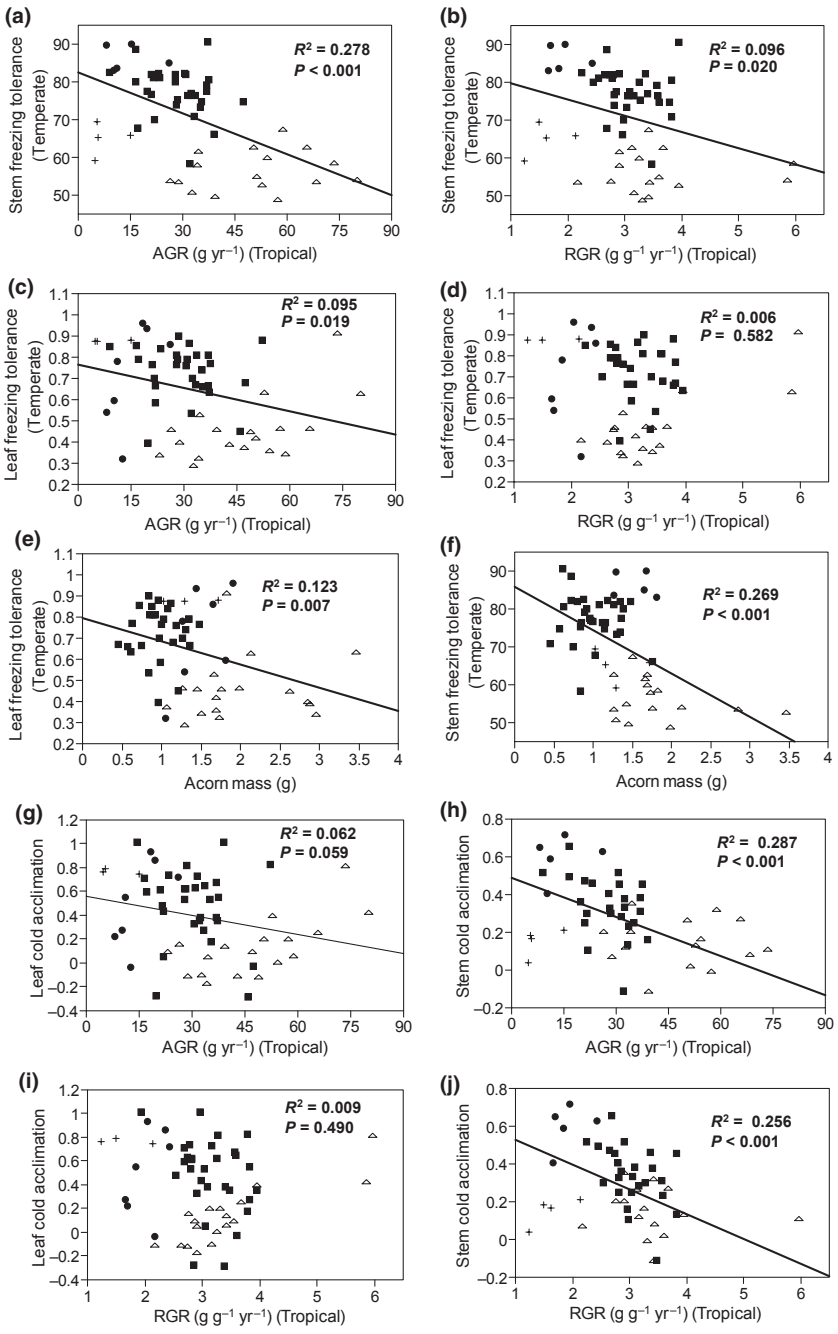

Fig. 4 Trade-offs between growth rate in nonstressed tropical conditions and freezing tolerance after cold acclimation in temperate conditions across maternal families from four live oak species: *Quercus virginiana* (squares), *Q. geminata* (crosses), *Q. fusiformis* (circles) and *Q. oleoides* (triangles). Points represent family means. Relationships are shown for: temperate stem freezing tolerance (100 − index of injury after freezing at − 15°C) and tropical absolute growth rate (AGR) (a); temperate stem freezing tolerance and tropical relative growth rate (RGR) (b); temperate leaf freezing tolerance (1 − decline in F_v/F_m after freezing at − 10°C) and tropical AGR (c); temperate leaf freezing tolerance and tropical RGR (d); temperate leaf freezing tolerance and acorn mass (e); temperate stem freezing tolerance and acorn mass (f); leaf cold acclimation ability ((tropical − temperate)/tropical for decline in F_v/F_m after freezing at − 10°C) and AGR under tropical conditions (g); stem cold acclimation ability ((tropical − temperate)/tropical for index of injury after freezing at − 15°C) and AGR under tropical conditions (h); leaf cold acclimation ability and RGR under tropical conditions (i); and stem cold acclimation ability and RGR under tropical conditions (j). Least squares fitted lines are shown for significant relationships ($P < 0.05$); the dashed line (g) indicates marginal significance. Corrections are not made for multiple tests.

correlated with AGR. Cold acclimation ability of stems (Fig. 4j), but not leaves (Fig. 4i), was negatively correlated with RGR.

The minimum temperature of the coldest month of the source populations predicted freezing tolerance, growth and cold acclimation (Fig. 5a–h). In ANCOVA, AGR significantly predicted stem freezing tolerance ($P = 0.012$, Table 2) when taking into account population- and species-level variations, and minimum temperature significantly predicted leaf freezing tolerance when taking into account species- and population-level variations ($P = 0.0136$, Table 2). Minimum temperatures in the coldest month also predicted seed mass (Fig. 5e), a relationship driven largely by the fact that the tropical species, *Q. oleoides*, had larger seeds than the species that occurred in temperate regions. Given that seeds were collected in the field, we cannot eliminate an environmental influence on seed mass. However, variation in seed mass was greater between than within species, despite steep environmental gradients within species ranges, suggesting that seed mass may be genetically constrained at the species level, as concluded in earlier studies (Baskin *et al.*, 1998; Morin & Chuine, 2006).

Discussion

We provide evidence for a trade-off between growth rate and freezing tolerance across maternal families from four species of live oak (*Quercus* series *Virentes*) that span the tropical–temperate divide across North and Central America. Maternal families with

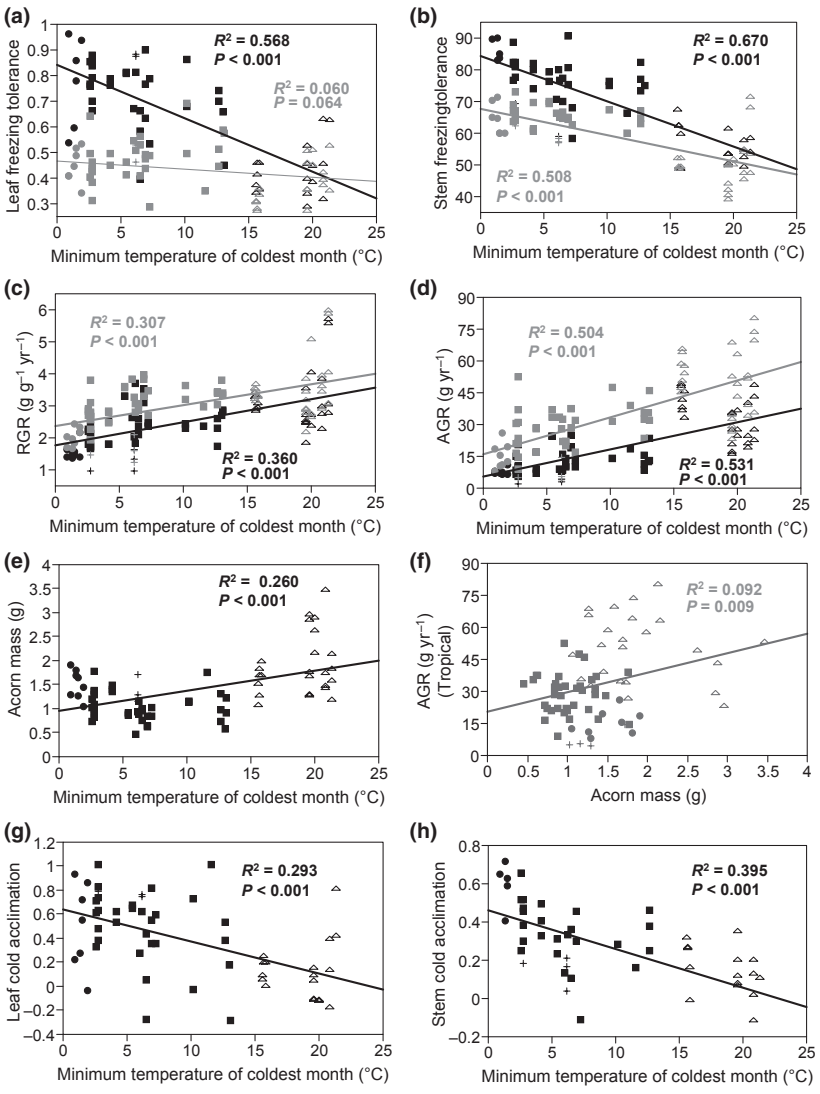

Fig. 5 Minimum temperature of the coldest month in the climate of origin predicts leaf (a) and stem (b) freezing tolerance and relative growth rate (RGR) (c) and absolute growth rate (d) under both tropical (gray) and temperate (black) growth conditions in maternal families from four live oak species. Minimum temperature of the coldest month also predicts acorn mass (e), and acorn mass is correlated with AGR under tropical (nonstressed) conditions (f). Minimum temperature of the coldest month further predicts leaf cold acclimation ability ((tropical − temperate)/tropical for decline in F_v/F_m after freezing at − 10°C) (g) and stem cold acclimation ability ((tropical − temperate)/tropical for index of injury after freezing at − 15°C) (h). Points represent family means. *Quercus virginiana*, squares; *Q. geminata*, crosses; *Q. fusiformis*, circles; *Q. oleoides*, triangles. Least squares fitted lines are shown for tropical (black) and temperate (gray) treatments. The dashed gray line (a) indicates marginal significance. Corrections are not made for multiple tests.

higher freezing tolerance under temperate (cold-acclimating) conditions had slower growth rates under tropical (nonstressed) conditions. These relationships were strongest between stem freezing tolerance (− 15°C) and both AGR and RGR, but were also supported for leaf freezing tolerance (− 10°C) and AGR (Fig. 4a–d). Unlike maternal families from *Q. oleoides*, families from temperate latitudes (from *Q. virginiana*, *Q. geminata* and *Q. fusiformis*) demonstrated the ability to increase freezing tolerance in response to chilling growth temperatures. The ability to cold acclimate and express higher freezing tolerance under temperate conditions than under tropical conditions was associated with less competitive growth rates under tropical (nonstressed) growth conditions (Figs 4g–j, S3). Lower AGRs in live oaks from more northern latitudes were driven by both lower RGR (Fig. S4a) and reduced seed mass (Fig. 5f). Seed mass declined with lower minimum temperatures (Fig. 5e) and may be limited by the length of the growing season because of energy constraints (Morin *et al.*, 2007b; Chuine, 2010). Our results indicate that, at more northern latitudes, live oaks have been selected to invest in freezing tolerance and cold acclimation ability, rather than higher growth rates and larger seed sizes, which may enforce species boundaries across the tropical–temperate divide. This trade-off supports the classic hypothesis that the distributions of species are limited by freezing tolerance in the north and by competition in the south (MacArthur, 1972; Woodward, 1987; Schenk, 1996; Gross & Price, 2000).

Climate of origin predicts growth rates, seed mass, freezing tolerance and cold acclimation ability

Live oak species showed significant differentiation in their sensitivity to freezing, as expected from their climatic distributions (Fig. 2a,b). Under both tropical and temperate growth conditions, minimum temperatures in the climate of origin strongly predicted freezing tolerance, growth rates, seed mass and cold acclimation ability of maternal families (Fig. 5). Maternal families from climates with colder winters had slower growth rates and greater freezing tolerance than those from milder climates. Minimum temperature was a significant covariate predicting leaf freezing tolerance when species, population and treatment were

Table 2 ANCOVA indicating that the absolute growth rate (AGR), relative growth rate (RGR) and minimum temperature are important factors predicting leaf and stem freezing tolerance within and among four live oak (*Quercus* series *Virentes*) species

	Leaf freezing tolerance				Stem freezing tolerance			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
Species	**3**	**0.4794**	**16.8472**	**< 0.0001**	**3**	**4990.5651**	**69.1223**	**< 0.0001**
Population (species)	7	0.1190	1.7931	0.0983	**7**	**418.0152**	**2.4813**	**0.0224**
Growth temperature treatment	**1**	**0.8596**	**90.6255**	**< 0.0001**	**1**	**1888.68**	**78.4781**	**< 0.0001**
Species × treatment	**3**	**0.3203**	**11.2573**	**< 0.0001**	**3**	**540.1189**	**7.481**	**0.0002**
Population (species) × treatment	**7**	**0.1451**	**2.1858**	**0.0427**	7	294.8277	1.7501	0.1074
AGR (g yr^{-1})	1	0.0224	2.3635	0.1277	**1**	**159.9096**	**6.6445**	**0.0116**
Species	**3**	**0.6761**	**23.483**	**< 0.0001**	**3**	**6948.3852**	**92.7592**	**< 0.0001**
Population (species)	7	0.0968	1.4421	0.1985	**7**	**450.5568**	**2.5778**	**0.0181**
Growth temperature treatment	**1**	**0.8856**	**92.2738**	**< 0.0001**	**1**	**1837.9017**	**73.6066**	**< 0.0001**
Species × treatment	**3**	**0.3524**	**12.2416**	**< 0.0001**	**3**	**649.2259**	**8.667**	**< 0.0001**
Population (species) × treatment	*7*	*0.1399*	*2.0827*	*0.0534*	*7*	*351.2304*	*2.0095*	*0.0624*
RGR (g g^{-1} yr^{-1})	1	0.0123	1.2835	0.2603	*1*	*78.6481*	*3.1498*	*0.0793*
Species	**3**	**0.1585**	**5.8095**	**0.0011**	**3**	**793.0926**	**10.2641**	**< 0.0001**
Population (species)	**7**	**0.1555**	**2.4427**	**0.0244**	7	362.9994	2.0134	0.0619
Growth temperature treatment	**1**	**0.9404**	**103.415**	**< 0.0001**	**1**	**1785.6148**	**69.3276**	**< 0.0001**
Species × treatment	**3**	**0.3404**	**12.4771**	**< 0.0001**	**3**	**610.3843**	**7.8995**	**< 0.0001**
Population (species) × treatment	**7**	**0.1472**	**2.3134**	**0.0324**	7	365.9245	2.0296	0.0598
Minimum temperature	**1**	**0.0576**	**6.3377**	**0.0136**	1	7.8229	0.3037	0.5829

Significant values ($P < 0.05$) are shown in bold and marginally significant values ($P < 0.1$) are shown in italic. SS, sum of squares.

included in the model (Table 2). Within *Q. virginiana*, the species with the greatest minimum temperature range, significant variation among populations was detected in stem and leaf freezing tolerance, as well as in AGR and RGR (Fig. 3b–j), suggesting the local adaptation of populations to latitudinal variation in climate. Within *Q. oleoides*, the Mexican population also showed greater freezing tolerance and ability to cold acclimate than populations from Belize or Costa Rica (Fig. 3f). The Mexican source populations encounter mild freezing temperatures in the winter months, whereas the rest of the range of *Q. oleoides* does not. Fewer collection sites for *Q. geminata* and *Q. fusiformis* limited our ability to detect population-level variation in these species.

The minimum temperature of the source population also strongly predicted cold acclimation ability across maternal families in a clinal pattern (Fig. 5e,f). Under tropical growth conditions with no cold acclimation, there was lower tolerance of stem and leaf freezing across all species and populations, although it was lowest in the tropical species *Q. oleoides* (Fig. 3c–f). The most northern families (from North Carolina) showed the highest cold tolerance and cold acclimation ability. An increase in cold acclimation ability at more northern latitudes (Fig. 5e,f) provides evidence for adaptive plasticity in response to the onset of winter temperatures. However, the variation in freezing tolerance and cold acclimation within species was low relative to the variation among species, suggesting that the distributions of species may be more strongly driven by broad climatic tolerances than by local adaptation.

Seed mass also increased with minimum temperature of the coldest month (Fig. 5e). Minimum temperature is strongly associated with season length and the total energy available for photosynthesis during the growing season. Both reproductive output and seed mass have been predicted to increase with season length (Chuine, 2010), and empirical evidence in forest trees indicates that seed mass decreases with latitude (Morin *et al.*, 2007b). Seed mass was positively associated with AGRs (Fig. 5f) and probably contributes to competitive ability.

Previous studies have shown a similar pattern of cold and freezing tolerance varying with latitude. Linear regressions for freezing and minimum temperature for populations of three oak species, *Q. ilex*, *Q. robur* and *Q. pubescens*, were significant at both the inter- and intraspecific levels (Morin *et al.*, 2007a). In *Betula pendula*, the northern-most ecotype was more responsive than the southern ecotype to cold acclimation and freezing tolerance (Li *et al.*, 2002). In *Cornus stolonifera*, more northern populations showed greater acclimation ability and cold resistance (Smithberg & Weiser, 1968). *Tamarix* and *Populus* populations showed latitudinal variation for cold hardiness and survival (Friedman *et al.*, 2008). Herbaceous species, such as *Arabidopsis*, also exhibit clinal variation in freezing tolerance (Zhen & Ungerer, 2008). Previous studies have also shown that minimum temperature predicts growth rate (Li *et al.*, 1998, 2002; Rehfeldt *et al.*, 2001; Green, 2007; Gimeno *et al.*, 2009; Savage, 2010). In a Mediterranean evergreen oak species, *Q. ilex*, grown under common conditions, individuals from more northern latitudes had lower growth rates, and clinal variation occurred between populations (Gimeno *et al.*, 2009).

Contrasting freezing tolerance strategies

We saw several different freezing tolerance strategies in the temperate species, which have overlapping climate distributions (Fig. 2a). In broad-leaf evergreen trees that replace leaves on an annual basis (leaf exchangers, *sensu* Givnish, 2002), early

senescence and leaf abscission in response to chilling represent a strategy to avoid freezing damage. Alternatively, leaves can be maintained for longer, but have higher freezing tolerance. We found evidence of evolution towards early leaf abscission in both *Q. virginana* and *Q. fusiformis*, with accelerated leaf loss after cold exposure in temperate conditions relative to leaf loss in tropical conditions. By contrast, *Q. geminata* maintained its leaves throughout the duration of the winter period under temperate conditions (Fig. 3a), but had higher leaf freezing tolerance (Fig. 3c). *Quercus geminata* and *Q. virginiana* co-occur from North Carolina to central Florida, where they experience similar winter temperatures in their local environments. *Quercus virginiana* apparently shows greater investment than *Q. geminata* in protecting its stem tissue from freezing damage, whereas the latter maintains its leaves under cold conditions and shows greater investment in protecting them from freezing damage. The variation in response to freezing may be explained by the ecological niche differentiation observed in *Q. virginiana* and *Q. geminata* (Nixon, 1985; Cavender-Bares & Pahlich, 2009). These sympatric species show niche differentiation across soil types, with *Q. virginana* found in moister, nutrient-rich soils and *Q. geminata* found in drier, nutrient-poor sites (Cavender-Bares *et al.*, 2004). Morphological data support the differentiation between the species, as *Q. virginiana* allocates a greater proportion of its resources to shoots and has larger and thinner leaves, whereas *Q. geminata* invests less in shoots and has smaller and denser leaves (Cavender-Bares & Pahlich, 2009). Although denser leaves may have adapted in response to dry habitat conditions, dense leaves are also an important attribute in cold-tolerant plants (Loehle, 1998).

Explaining trade-offs between growth and freezing tolerance

In order for a trade-off to be biologically important, a fitness advantage in one environment would result in a lower fitness in a contrasting environment, and vice versa. Greater stem and leaf freezing tolerance after cold acclimation presumably provides a fitness advantage in seasonal climates that incur winter freezing. Greater accumulated biomass under year-round warm conditions presumably provides a fitness advantage in the tropics. There is no selective advantage to investing in high freezing tolerance in the absence of freezing stress, and actively growing tissues are inherently vulnerable to freezing (Howe *et al.*, 2003; Li *et al.*, 2003). As freezing temperatures become more severe and the duration of winter increases at higher latitudes, natural selection would be expected to cause an increase in freezing tolerance in plants and the ability to cold acclimate. Explaining the reduced growth in colder climates is somewhat more complicated, and there are likely to be multiple contributing factors. In particular, two explanations are supported by the current study. The first is that resource allocation to freezing tolerance limits allocation to growth. A number of biochemical changes occur during cold acclimation that confer freezing tolerance, including an increase in saturated lipids, the accumulation of water-soluble solutes and conformational changes in cell membranes (Levitt, 1980; Beck, 1988). In addition, structural investments, such as the development of thicker leaves, are important for cold acclimation (Chabot & Hicks, 1982; Körner & Larcher, 1988). Resource investment in physiological and structural traits for cold and freezing tolerance is thus hypothesized to cause more conservative growth. The lower growth rates (RGR and AGR) in source locations with colder winters, even under nonstressed, tropical conditions (Fig. 5c,d), and in maternal families with higher freezing tolerance and cold acclimation ability, support this explanation.

A second potential contributing factor to slower growth is that a shorter season length at northern latitudes limits reproductive output and seed size as there is less time for carbon accumulation (Morin & Chuine, 2006; Chuine, 2010). Reduced seed size may then drive lower AGRs. Consistent with this explanation, seed mass is reduced in climates with colder winters (Fig. 5e), where the growing season is presumably shorter, and seed size is correlated with AGR (Fig. 5f). The latter relationship is significant, although the variation explained is low: $R^2 = 0.092$, $P = 0.009$. Therefore, seed mass may be contributing directly to the trade-off between AGR and freezing tolerance. However, reduced seed mass cannot explain the negative relationship between stem freezing tolerance and RGR ($R^2 = 0.29$, $P < 0.001$, Fig. 4j), because seed mass is not correlated with RGR (Fig. S4b).

The strongest relationships between freezing tolerance and growth are found when AGR is the measure of growth (Fig. 4; Table 2). However, RGR and AGR are strongly positively correlated ($R^2 = 0.69$, $P = 0.001$, Fig. S4a). Both RGR and seed mass, which are both positively correlated with AGR and minimum temperatures, but negatively correlated with stem freezing tolerance, thus contribute to the trade-off between freezing tolerance and AGR. It is interesting to note that leaf freezing tolerance is negatively associated with seed mass and AGR, but not with RGR (Fig. 4c–e). RGR may be less likely than AGR to trade off with freezing tolerance, because rapid growth rates may be required in short growing seasons. Alternatively, slower growth in cold climates may be adaptive, if a more conservative growth strategy limits the risk of tissue damage (Guy, 1990). Given the limits to carbon accumulation with a shorter season length, season length may drive selection for both lower seed mass and lower AGR; however, the smaller seed, slower growth 'strategy' may also be a consequence of resource allocation to cold tolerance (Lambers *et al.*, 2008).

Life history strategies and limits to range expansion

Low temperatures are a major limitation to northern range expansion in many species that lack freezing tolerance (Sakai & Weiser, 1973; Ricklefs *et al.*, 1999). The lack of cold acclimation ability and freezing tolerance exhibited by *Q. oleoides* indicates a physiological barrier that probably prevents expansion beyond its northern range limit. The extremely limited migration of *Q. oleoides* northwards over the last 1–2 million yr is supported by molecular evidence (Cavender-Bares *et al.*, 2011). If growth rate is an indicator of relative fitness and competitive ability (Lambers *et al.*, 2008; Moles *et al.*, 2009), the growth rate–freezing tolerance trade-off also implies that southern expansion of the

temperate species, *Q. virginiana*, *Q. geminata* and *Q. fusiformis*, may be limited by their inferior competitive ability relative to the tropical species, *Q. oleoides*, or relative to other species at or below their southern range limits.

Species generally have maximum growth rates at their southern range limit, but do not increase their growth rates when grown further south or under optimal conditions (Langlet, 1971; Sakai & Larcher, 1987; Roberds *et al.*, 1990; Loehle, 1998). This is true for the temperate species *Q. fusiformis* and *Q. geminata*, but was more variable for *Q. virginiana*. Under year-round tropical conditions, populations of the temperate species *Q. geminata* and *Q. fusiformis* maintained lower growth rates and were unable to increase growth rates to a level comparable with that of tropical *Q. oleoides* (Fig. 3g,i). However, some maternal families and one population of *Q. virginana* (Louisiana) had AGRs similar to that of *Q. oleoides* under tropical conditions (Fig. 3h,j). This suggests that, although *Q. geminata* and *Q. fusiformis* may have limited ability to increase their growth rates under tropical conditions, some *Q. virginiana* populations may achieve competitive growth rates. There was much greater overlap in RGR than in AGR values, such that regressions with RGR as the predictor of freezing tolerance were less significant (Fig. 4a–d). The total biomass achieved by a plant within a given period of time (AGR) may ultimately be the best predictor of fitness and/or competitive ability. Seed mass is also associated with competitive ability and contributes directly to AGR. However, other factors not investigated, including susceptibility to biotic pathogens and pests (Reader, 1992; Alexander & Holt, 1998; Meiners & Handel, 2000; Fine *et al.*, 2004), are thought to play an important role in mediating competition and limiting species ranges. Seasonal drought that occurs throughout the range of *Q. oleoides* in Central America may also interact with temperature to mediate species ranges. Asymmetrical gene flow between *Q. oleoides* and *Q. virginiana*, with higher rates southwards than northwards (Cavender-Bares *et al.*, 2011), suggests that a lack of freezing tolerance may present a more formidable barrier to northward expansion than does the lack of competitive ability to southward expansion.

Local adaptation or broad climatic tolerances?

Across the latitudinal gradient of the live oak group, we found limited variation among populations, suggesting conserved species-level climatic tolerances. In *Q. virginiana*, the northern-most population of North Carolina exhibited greater leaf and stem freezing tolerance than the Florida populations, and mean values of the populations suggested clinal variation along the latitudinal gradient (Fig. 3d,f). A previous but more limited study with *Q. virginiana* found similar differentiation between the North Carolina and Florida populations for leaf freezing tolerance, indicating that the population-level differences are repeatable, even across different experimental conditions and collection locations (Cavender-Bares, 2007). Minimum temperature is a good predictor for freezing tolerance across maternal families (Fig. 4e,f), a relationship driven by both the variation among species and within *Q. virginiana* and, to a lesser extent, within *Q. oleoides*. Within the broadly distributed species, *Q. virginiana* and *Q. oleoides*, variation among maternal families followed a latitudinal trajectory, showing decreased freezing tolerance with increased growth rate (Fig. 3d,f,h,j). These results suggest some degree of local adaptation to freezing, particularly within *Q. virginiana*. Local adaptation within *Q. virginiana* must have occurred since the Pleistocene, when *Q. virginiana* and *Q. oleoides* are thought to have diverged from a common ancestor (Cavender-Bares *et al.*, 2011). A study with *Quercus suber* found population-level differentiation under winter conditions based on the annual temperature of the population source (Aranda *et al.*, 2005). Other studies support the local adaptation hypothesis and show population-level variation in cold sensitivity corresponding to habitat of origin (Rehfeldt *et al.*, 2002). Clinal variation in freezing tolerance has been demonstrated in several tree species, indicating local adaptation (Ducousso *et al.*, 1996; Rehfeldt *et al.*, 2001; Aranda *et al.*, 2005; Cavender-Bares, 2007; Morin *et al.*, 2007a).

Nevertheless, the trade-off occurred largely across species (Fig. 4a–d; Table 1), such that temperate species exhibited slower growth rates and higher levels of freezing tolerance, whereas the tropical species maintained higher growth rates and exhibited greater leaf and stem damage in response to freezing. The limited variation among populations, compared with the variation among species, in freezing tolerance (Table 1) indicates conservatism in the climatic niches of species. This finding is consistent with limited local adaptation in populations of Mediterranean holm oak (Gimeno *et al.*, 2009). Conserved climatic niches and broad climatic tolerances may thus be more important than the local adaptation of populations in determining species ranges. Across the temperate and tropical divide, we see a distinct break, as evidenced by the reduced ability of *Q. oleoides* to cold acclimate and avoid freezing damage. This corresponds with the narrow range of minimum temperatures of *Q. oleoides* when compared with the three more temperate species exhibiting overlapping temperature ranges (Fig. 2a). Both *Q. virginiana* and *Q. fusiformis* have a fairly broad range of minimum temperature climatic tolerances (Fig. 2a). Temperate species that encounter high climatic variability throughout the year may be adapted to tolerate a wide range of climatic conditions and thus occupy broad latitudinal and climatic distributions with limited local adaptation (Janzen, 1967; Larcher, 2005; Morin & Chuine, 2006).

Conclusions

The trade-off between freezing tolerance and growth rate supports the classic range limit hypothesis, which posits that the distributions of species are limited by freezing tolerance in the north and by competition in the south (MacArthur, 1972; Woodward, 1987; 1988; Schenk, 1996; Gross & Price, 2000). Species and populations from colder climates have a greater ability to enhance their freezing tolerance after exposure to cold temperatures than those from more tropical latitudes, providing evidence for adaptive plasticity. Freezing tolerance, cold acclimation ability and diminution of growth rates have probably

evolved since the split between *Q. oleoides* and the *Q. virginiana* clade, which is estimated to have occurred in the mid-Pleistocene (Cavender-Bares *et al.*, 2011). The freezing tolerance–growth rate trade-off is likely to be critical in limiting species range expansion northwards, minimizing secondary contact across the tropical–temperate divide.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Predicted species range for four live oak species based on precipitation and temperature.

Fig. S2 Leaf decline in F_v/F_m after freezing (− 10°C) and stem freezing injury at five freezing temperatures for temperate and tropical growth treatment.

Fig. S3 Regressions for leaf and stem freezing tolerance and absolute growth rate (AGR) and relative growth rate (RGR) for both temperate and tropical treatments.

Fig. S4 Mean acorn mass for species and populations.

Table S1 Acorn collection site locations for all maternal families

Table S2 Restricted maximum likelihood (REML) variance component estimates for all response variables for the amount of variation explained by species and population for each growth temperature treatment

Methods S1 Leaf and stem freezing method details.

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