Convergence of three mangrove species towards freeze-tolerant phenotypes at an expanding range edge

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Summary

1. Climate change is dramatically altering the distribution and abundance of many species. An examination of traits may elucidate why some species respond more strongly to climate change than others, particularly when ecophysiological thresholds set range limits.

2. Mangrove forests are expanding polewards. Although multiple environmental factors influence mangrove distributions, freeze tolerance is hypothesized to determine their poleward extent. To investigate how trait variation influences mangroves’ responses to a warming climate, we examined how freeze tolerance and associated traits varied along a latitudinal cline for three co-occurring mangrove species.

3. We sampled individuals along >200 km of Florida, USA’s eastern coast, from the mangroves’ most northern populations, where freeze events were historically common, to southern populations where freeze events continue to be rare.

4. We measured a suite of traits in field-collected adults and their garden-reared offspring, and assessed their responses to an experimentally imposed freeze event. We asked whether freeze tolerance and other traits varied predictably among species, with latitude, and between life stages.

5. Species and populations varied dramatically in freeze tolerance, with the highest freeze tolerance in the northernmost species and populations, and the lowest freeze tolerance in the southernmost species and populations. Additionally, leaves of all three species were drier, tougher, thicker and more freeze-tolerant at the range edge.

6. Tolerance to freezing appears to set the range limits for these mangrove species. All three species converged on a similar phenotype at the range edge, but species-level variation in freezing resistance was conserved. Thus, these species are likely to continue migrating at different rates in response to climate warming, potentially leading to the dissolution of typically co-occurring species and creating ‘no analogue’ coastal mangrove-marsh communities.

Key-words: Avicennia germinans, climate change, freeze tolerance, Laguncularia racemosa, latitudinal limits, mangrove, Rhizophora mangle

Introduction

The range limit hypothesis posits that freezing stress limits the poleward distributions of species (MacArthur 1972), and indeed, the distributions of many species are closely aligned with their freezing tolerances (Shreve 1914; Sakai & Weiser 1973). However, as the climate warms, many species distributions are shifting. While the general movement is towards higher elevations and latitudes, co-occurring species can differ dramatically in where they are moving and at what rate (Burrows et al. 2011; Chen et al. 2011; Buckley & Kingsolver 2012). Interspecific variation in key ecophysiological traits can help explain these differential responses to climate change (Buckley & Kingsolver 2012), particularly if species’ geographic distributions are closely linked to physiological tolerance of abiotic factors such as freezing.

Organism traits, however, are strongly context-dependent and can vary among populations across a species’ range due to plasticity or to novel selective pressures (Suarez & Tsutsui 2008; Alberto et al. 2013; McCann et al. 2014). For example, different tree species exhibit clinal variation in freeze tolerance (Rehfeldt, Wykoff & Ying 2001; Aranda et al. 2005; Morin et al. 2007; Koehler, Center &
Cavender-Bares 2011), indicating local trait adaptation to freezing temperatures and suggesting a complex, population-level response to climate change. Thus, careful analyses of how traits and abiotic factors change along key climatic gradients can illuminate the mechanisms setting range limits (Kearney & Porter 2009) and indicate how climate change will impact species and populations. Analyses of traits along a species range can also inform predictive modelling of future range limits, which do not often account for population-level variation in thermal performance (Angert, Sheth & Paul 2011).

Mangroves are a useful system for studying trait-driven responses to climate change. Occupying a narrow environmental niche along tropical and subtropical coasts (Spalding, Kainuma & Collins 2010; Buckley & Kingsolver 2012), mangroves are expanding their ranges around the globe (Sherrod & McMillan 1981; Sainiljan et al. 2013; Cavanaugh et al. 2014; McCann et al. 2014) and contributing to the growing predominance of tropical species in temperate zones (Wernberg et al. 2013; Verges et al. 2014). Mangrove distributions are thought to be controlled by a combination of salinity, drought, inundation and freezing tolerance (Duke, Ball & Ellison 1998), but many hypothesize that freeze tolerance is especially important at the poleward extent of mangroves (Markley, McMillan & Thompson 1982; Sherrod & McMillan 1985; Soares et al. 2012). Indeed, a recent analysis found a strong relationship between mangrove expansion in Florida and the frequency of extreme cold events below −4 °C (Cavanaugh et al. 2014), suggesting that freeze tolerance traits dictate mangrove distributions at the range edge.

Surprisingly few studies, however, have experimentally examined freeze tolerance in mangroves despite the widespread belief that it is a range-limiting factor (Markley et al. 1982; Stuart et al. 2006; Pickens & Hester 2011). Instead, most examinations of mangrove freeze tolerance are either field observations of damage after uncontrolled freeze events (Sherrod & McMillan 1985; Ross et al. 2009; Wang et al. 2011) or observations of declining mangrove density and diversity towards the poles (Hsueh & Lee 2000). To our knowledge, there are only two experimental tests of how freeze tolerance varies among or within mangrove species. Stuart et al. (2006) observed that xylem diameter size was linked to freeze tolerance in five Florida and Australian species. They noted that the more geographically restricted species in North America had larger vessel diameters than the three more poleward Australian species, suggesting that trait variation may shape range limits. Markley, McMillan and Thompson (1982) raised mangrove seedlings in a common garden at the northern range edge and found that for two of the three species, the southern populations had lower survival. Taken together with broader surveys of mangrove climatic distributions (Duke, Ball and Ellison 1998), these results suggest that freezing temperatures shape mangrove range limits. However, to date, there is no evidence that interspecific variation in freeze tolerance sets range limits for mangrove species within the same climatic zone and only limited evidence for clinal variation in mangrove freeze tolerance.

We sampled three co-occurring mangrove species (Fig. 1) along more than 200 km of the eastern Florida coast where a strong latitudinal cline in temperature occurs. We experimentally examined whether species, populations and life stages varied predictably in freeze tolerance and whether traits could explain this variation. We tested three hypotheses: (i) species will vary in freeze tolerance, (ii) freeze tolerance will vary with latitude, and (iii) trait variation will be linked to freeze tolerance. We predicted that the most northerly distributed species and

![Fig. 1. Study organisms with (a) black mangrove leaves and flowers, (b) full red mangrove tree and (c) white mangrove leaves depicted (photograph credits: JD Parker).](image)

populations would be the most freeze-tolerant and the least northerly would have the least freeze tolerance.

**Materials and methods**

Mangroves are defined by similar adaptations to harsh saline environments rather than phylogenetic relationships (Spalding, Kainuma and Collins 2010). They occupy much of the world’s tropical and subtropical coastlines (Spalding, Kainuma and Collins 2010) and provide habitat for many animal species, as well as trillions of dollars in ecosystem services (Costanza et al. 1997; Hogarth 1999). There are over 70 different mangrove species around the globe with varying poleward extents (Spalding, Kainuma and Collins 2010). In Florida, three species of mangroves co-occur: Avicennia germinans, Rhizophora mangle and Laguncularia racemosa, hereafter referred to as black, red and white mangroves, respectively. Although these three species largely co-occur throughout most of their range, there is evidence that they are migrating northwards at different rates. The northernmost black mangrove is currently found at 30°11′ N, 81°37′ W; the northermest red mangrove is at 29°94′ N, 81°32′ W; and the northernmost white mangrove is at 29°73′ N, 81°24′ W (Williams et al. 2014). Historical observations of black mangroves (from 2007), red mangroves (2006) and white mangroves (from 1962) suggest migration rates of 4.5, 3.7 and 1.3 km/year, respectively (Williams et al. 2014).

We sampled mangroves at 12 different sites (Table 1). The northernmost site represents the current limit of mangroves in the United States (Williams et al. 2014) and experiences a mangrove-killing, freeze event (−4°C or colder; Cavanaugh et al., 2014) every three years on average. The southernmost site has not experienced a deep freeze event since 1985 (http://climatecenter.fsu.edu/climate-data-access-tools/downloadable-data; northern data: Jacksonville weather station, 42.5 km from nearest focal tree; southern data: Stuart weather station, 9 km from nearest focal tree). Thus, there is a robust relationship between latitude and mean coldest monthly temperature (R² = 0.96; PRISM climate data, 1980–2011). In contrast, salinity did not vary widely among sites (Table 1; 34.7 ppt ± 4.2; mean ± SD) or systematically with latitude (R² < 0.1).

### FREEZE EXPERIMENT – ADULT SAMPLES

At each site, we selected up to ten focal adults per species (Table 1), depending on availability. On average, trees were 40 m apart and sites were 30 km apart. In October 2012 and January 2013, we collected adult samples from 98-well trays and placed them in a shaded, outdoor pond (Smithsonian Marine Station, Fort Pierce, FL; 27°45′ N, −80°31′ W). The pond contained sea water ~15 cm deep (35 ppt salinity) that we replaced every 7–10 days with water collected 1.6 km offshore.

In mid-January 2013, after propagules had rooted, we randomly assigned half of the seedlings per parent to the same freeze treatment as the adults (N = 1106 or 3·5 seedlings/parent); the remaining control seedlings remained on a laboratory countertop during the freeze treatment (N = 865 or 2·9 seedlings/parent). Because soil temperatures remained above freezing (17°C ± 2·1°C with average minimums of 12·7°C ± 2·3°C, mean ± 1 SD, N = 28 trials), only the above-ground portion of the seedlings experienced freezing temperatures. We then returned seedlings to the outdoor pond where no natural freezing events occurred before the destructive harvest in June 2013. Daily minimums ranged from 1·1°C to 25·0°C between mid-January 2013 and mid-June 2013 (mean 15·4°C; St. Lucie Airport weather station, ~5 km distant from the pond).

### FREEZE TOLERANCE

Our metrics of freeze tolerance (where freeze tolerance equals the ability of individuals to maintain function after a freeze event) while holding the cut end under water and placed these segments directly into floral water tubes. We brought branches to the laboratory, where one served as a control and the other was frozen for 4 h. We set the chest freezers at −4°C, but temperatures fluctuated as the compressor cycled, and temperature loggers revealed that the mean temperature across trials was −2·6°C ± 1·7°C with average minimums of −3·2°C ± 1·9°C (mean ± 1 SD). Thus, our minimum temperatures overlapped with −4°C, a temperature identified as a critical threshold in Florida (Cavanaugh et al. 2014). In comparison with natural conditions at the range edge, pilot data from in situ monitoring (29°72′ N, −81°24′ W) and data from WeatherSource.com (St. Augustine Airport) indicated that the average time at the minimum temperatures (−2.3°C ± 0.4°C) was 3·9 h in 2009–2014.

**Table 1.** The 12 sites employed in the study with latitude, winter salinity values collected from water surface and sample sizes for each mangrove species at each site. Note, not all species were present at the northern sites and at Guana Tolomato Matanzas, we sampled every available red mangrove

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Salinity</th>
<th>Avicennia germinans</th>
<th>Laguncularia racemosa</th>
<th>Rhizophora mangle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guana Tolomato Matanzas – N</td>
<td>29.875</td>
<td>36.5</td>
<td>10</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Guana Tolomato Matanzas – S</td>
<td>29.680</td>
<td>38.3</td>
<td>10</td>
<td>10</td>
<td>7</td>
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<tr>
<td>Gamble Rogers</td>
<td>29.439</td>
<td>35.5</td>
<td>10</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tomoka Beach</td>
<td>29.352</td>
<td>24.8</td>
<td>10</td>
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<td>–</td>
</tr>
<tr>
<td>New Smyrna Beach</td>
<td>29.087</td>
<td>36.0</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Merritt Island</td>
<td>28.709</td>
<td>40.0</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Pine Island</td>
<td>28.491</td>
<td>38.0</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Indian River Lagoon</td>
<td>27.948</td>
<td>30.0</td>
<td>10</td>
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<tr>
<td>Sebastian</td>
<td>27.875</td>
<td>35.0</td>
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<tr>
<td>Pelican</td>
<td>25.806</td>
<td>36.0</td>
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<tr>
<td>Avalon</td>
<td>27.551</td>
<td>31.0</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>St. Lucie</td>
<td>27.141</td>
<td>36.0</td>
<td>7</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>
included seedling survival, seedling biomass and photoinactivation. Survival equaled the number of seedlings that survived versus died per parent and treatment combination. Biomass equaled the dry weight of all root tissue and new above-ground growth for surviving seedlings, excluding the mass of the original propagule. Photoinactivation equaled the change in chlorophyll fluorescence yield (Y) after a freeze event and is a common freeze tolerance metric (Cavender-Bares et al. 2005; Pérèz et al. 2014) that correlates with other freeze tolerance metrics (Boorse et al. 1998). The yield of a light-adapted sample is \(Fm'-Fm'/Fm'\), where \(Fm'\) is the fluorescence before a saturation pulse is applied and \(Fm\) is the maximal fluorescence of a light-adapted sample with all photosystem II centres closed (Genty, Briantais & Baker 1989). We measured yield before and after the freeze treatment with a mini-PAM (Photosynthetic Yield Analyzer, Walz, Mass- und Regeltechnik, Germany) and quantified photoinactivation as \(1 - Y_{\text{post}}/Y_{\text{max}}\) (Pérèz et al. 2014). Photoinactivation thus ranged from zero (no loss of photosynthetic function) to one (complete loss of photosynthetic function). Because yield is sensitive to the light history of leaves, temperature and current light conditions, we sampled analogous leaves across individuals. For adults, we only utilized the youngest mature leaf on full-sun branches after they had sat in uniform light and temperature conditions (e.g. in a laboratory) for at least an hour prior to measurement and repeatedly sampled the same leaf location at the same orientation. For seedlings, we used individuals that had grown in uniform light conditions in the common garden, but otherwise followed the same protocol as for the adults.

OTHER TRAITS

To better understand how freeze tolerance might relate to other key traits, we measured xylem diameter (adults only), leaf osmolality, leaf size, leaf toughness, specific leaf area and leaf water content. Xylem diameter size is linked to salinity and water availability, but also freeze tolerance (Cavender-Bares et al. 2005; Stuart et al. 2006). We measured xylem diameters in control branches (mean branch diameter = 2.4 mm) that were collected in October 2012, air-dried, then rehydrated in water and sliced into transverse sections with a hand microtome. We photographed three sections per branch under 20x magnification and used ImageJ to record the diameter of 30 xylem vessels per individual to the nearest 0.1 mm. Vessels were elliptical, so we calculated the diameter of a circle with the same area and determined mean xylem diameter per individual. Osmolality is leaf solute concentration and can indicate freezing resistance, with higher solute concentrations reducing freeze damage (Scarth & Levitt 1937; Kostal et al. 2011). We removed a 29-mm² leaf disc from experimental branches before they were frozen, froze the discs in micro centrifuge tubes to lyse cells (−80°F, >24 h), added 20 µL of purified water and placed them on a shaker table for 20 h (140 rpm). We measured osmolality (Wescor 5500 Vapor Pressure Osmometer) with 10 µL of the resulting supernatant on a filter paper disc. We collected data from adults in October and January, and calculated the average. For seedlings, we measured osmolality in January only. Leaf size and specific leaf area (SLA; leaf area/dry mass) are affected by thermal and other environmental stressors (Buot & Okitsu 1999; Knight & Ackerly 2003), whereas leaf toughness and proportional water content (water content/wet weight) have been linked to herbivore resistance (Scriber 1977; Clissold et al. 2009). To measure these last traits, we collected the leaf closest to the one used to measure yield on the control branches and seedlings. We measured leaf size (Licor Model 2100 Area Meter, cm²), calculated the mean force required to puncture the leaf on either side of the midvein (Mecmesin Basic Force Gauge, N) and determined fresh and dried mass (Denver Instrument APX 323; 0.001 g).

Analyses

FREEZE TOLERANCE

We analysed all data with R (v3.0.1, R Foundation for Statistical Computing), conducting analyses separately for adults and seedlings except when we examined whether traits were correlated between adults and their offspring. We included site salinity as a covariate in all analyses because it can be correlated with ecophysiological traits (Tomlinson 1986; Duke, Ball and Ellison 1998; Spalding, Kainuma and Collins 2010). However, including salinity in the models never changed species-, latitudinal- or freeze-related results, so for simplicity, we do not discuss it further.

We first examined photoinactivation with logistic regression (glm in R package stats, R Core Team) with species, latitude, species × latitude as predictors. We followed with analyses by species, with latitude as the predictor. Because we observed very little photoinactivation in control samples (adults: 0.022 ± 0.002; seedlings: 0.037 ± 0.002; mean ± SE), we restricted photoinactivation analyses to frozen individuals. We also measured photoinactivation in both October and January for adults to ensure that we did not miss maximum freeze tolerance during the year. However, photoinactivation did not differ between months for blacks \(t = -1.63, P = 0.102\) or reds \(t = -1.85, P = 0.065\) and showed consistent patterns for whites \(t = -3.34, P < 0.001\); i.e. whites always had the greatest photoinactivation among species in both time periods), so we calculated mean photoinactivation per adult across time \((N = 296)\). For seedlings, we calculated mean photoinactivation among seedlings from each parent \((N = 189)\).

We analysed seedling survival using logistic regression, with species, latitude, treatment (control vs. freeze) and all two-way interactions as predictors \((N = 574; 290\) parents with frozen seedlings, 284 parents with control seedlings). We followed with analyses by species, with latitude, treatment and latitude x treatment as predictors. Finally, we used ANOVA \((\text{lm} \text{in R base package stats}; N = 574)\) and the same predictors to analyse mean seedling biomass per parent in each treatment.

OTHER TRAITS

We first employed a multivariate analysis of variance (MANOVA in R package stats) to control for correlations among traits. We had complete sampling of adult traits (i.e. leaf size, water content, specific leaf area, toughness, osmolality and xylem diameter) for 208 individuals \((N = 81\) blacks, 66 reds, 61 whites) and complete sampling of seedling traits (i.e. leaf size, water content, specific leaf area, toughness and osmolality) in 55 individuals \((N = 22\) blacks, 16 reds, 17 whites, all from different adult trees). We ln-transformed xylem diameter and included species, latitude and species x latitude as predictors.
All predictors for adults were significant (spp: Wilk’s \( \lambda = 0.26, \) \( P < 0.0001 \); latitude: Wilk’s \( \lambda = 0.65, \) \( P < 0.0001 \); species \( \times \) latitude: Wilk’s \( \lambda = 0.82, \) \( P < 0.0001 \)). We therefore followed up with ANOVAs on each trait with the same predictors and then conducted separate ANOVAs by species to see whether strong species-level differences obscured any latitudinal trends, with latitude as the only predictor. For seedlings, only species identity accounted for trait variation (Wilk’s \( \lambda = 0.06, \) \( P < 0.0001 \); latitude: Wilk’s \( \lambda = 0.8, \) \( P = 0.331 \); species \( \times \) latitude: Wilk’s \( \lambda = 0.7, \) \( P = 0.386 \)), so we used species as the only predictor in subsequent ANOVAs. Finally, to examine whether offspring and parents shared similar traits, we examined the correlations between parent and mean seedling trait values, conducting an analysis for each species and trait separately (\( N = 22 \) blacks, 16 reds, 17 whites).

Lastly, to determine how trait syndromes affected freeze tolerances, we centred and scaled all adult traits and collapsed them into principal components (prcomp in R package stats). We then examined the Pearson’s product-moment correlations between photoinactivation and the first two principal components for each species (cor.test in R package stats). To examine whether trait syndromes were similar among and within species, we constructed a hierarchical cluster diagram (hclust in R package cluster) with centred and scaled trait means for each species at the different sites.

**Results**

**HYPOTHESIS 1: SPECIES WILL VARY IN FREEZE TOLERANCE**

Photoinactivation after a freeze event differed by species for adults and seedlings (adults: deviance=2,293 = 1742.0, \( P < 0.0001 \); seedlings: deviance=2,186 = 2041.5, \( P < 0.0001 \)), with black mangroves showing the least susceptibility, followed by red and then white mangroves (Fig. 2a,c). Similarly, freezing increased seedling mortality (deviance=1,567 = 34.0, \( P < 0.0001 \)) and the effect varied by species with blacks showing the smallest change, followed by red and then white mangroves (Fig. 2c; species: deviance=2,569 = 257.5, \( P < 0.0001 \); species \( \times \) treatment: deviance=2,562 = 25.9, \( P < 0.0001 \)). Freezing had no effect on black seedling survival (\( \sim 85\% \) survival regardless of treatment; deviance=1,216 < 0.1, \( P = 0.824 \)), whereas freezing reduced red survival by 17\% (from 97\% to 80\%; deviance=1,147 = 45.5, \( P < 0.0001 \)) and white survival by 25\% (from 58\% to 43\%; deviance=1,173 = 14.5, \( P < 0.0001 \)). In contrast, freezing did not affect biomass of surviving seedlings for any species (\( R^2 \) for the full model = 0.44; treatment: \( F_{1,525} = 2.8, \) \( P = 0.091 \); species \( \times \) treatment: \( F_{2,525} = 0.1, \) \( P = 0.940 \)), although species did differ overall in biomass production with black mangroves growing the most, followed by red and then white mangroves (Fig. 2d; \( F_{2,525} = 211.81, \) \( P < 0.0001 \)).

**HYPOTHESIS 2: FREEZE TOLERANCE WILL VARY WITH LATITUDE**

Among adults, range edge populations were less susceptible to photoinactivation than southern populations (latitude: deviance=1,292 = 136.8, \( P < 0.0001 \), although this pattern was driven by black and red mangroves (Fig. 2a; species \( \times \) latitude: deviance=2,289 = 151.3, \( P < 0.0001 \); black: deviance=1,115 = 62.3, \( P < 0.0001 \); red: deviance=1,289 = 185.9, \( P < 0.0001 \); white: deviance=1,86 = 0.6, \( P = 0.433 \)). Seedlings showed the reverse trend, with black and white seedlings from the range edge showing increased susceptibility to photoinactivation (Fig. 2b; latitude: deviance=1,185 = 85.6, \( P < 0.0001 \); species \( \times \) latitude: deviance=2,182 = 71.3, \( P < 0.0001 \); black: deviance=1,87 = 77.2, \( P < 0.0001 \); red: deviance=1,38 = 0.5, \( P = 0.446 \); white: deviance=1,58 = 73.0, \( P < 0.0001 \)). For blacks, however, the effect was small, ranging from no damage in the south to less than 10\% photoinactivation in the north (Fig. 2b).

Black and red seedlings from the range edge had lower overall mortality than southern populations (Fig. 2c; latitude: deviance=1,568 = 3.7, \( P = 0.052 \); species \( \times \) latitude: deviance=2,564 = 7.5, \( P = 0.028 \); black: deviance=2,217 = 7.7, \( P = 0.005 \); red: deviance=1,175 = 4.7, \( P = 0.042 \); white: deviance=1,174 = 6.0, \( P = 0.408 \)). There was no overall effect of latitude on biomass production, because each species responded differently (Fig. 2d; latitude: \( F_{1,522} = 2.5, \) \( P = 0.112 \); latitude \( \times \) species: \( F_{2,522} = 10.0, \) \( P < 0.0001 \)).
Biomass accumulation increased at the range edge for blacks ($F_{1,202} = 4.3$, $P = 0.039$), decreased for reds ($F_{1,171} = 21.6$, $P < 0.0001$) and showed no latitudinal pattern for whites ($F_{1,148} = 0.4$, $P = 0.506$). In addition, the freeze treatment did not alter latitudinal trends in mortality (overall: deviance $F_{1,561} = 0.2$, $P = 0.611$; within species: $P > 0.7$) or biomass production (overall: $F_{1,325} = 0.4$, $P = 0.504$; within species: $P > 0.2$).

**HYPOTHESIS 3: TRAIT VARIATION WILL BE LINKED TO FREEZE TOLERANCE**

All adult traits (Appendix Table S1) and most seedling traits (except toughness) varied significantly among species (seedlings – leaf size: $F_{2,50} = 13.6$, $P < 0.0001$, $R^2 = 0.25$; water content: $F_{2,50} = 9.6$, $P < 0.001$, $R^2 = 0.25$; specific leaf area: $F_{2,50} = 17.5$, $P < 0.0001$, $R^2 = 0.43$; toughness: $F_{2,50} = 0.2$, $P = 0.745$, $R^2 = 0.04$; osmolality: $F_{2,50} = 24.4$, $P < 0.0001$, $R^2 = 0.47$). For adults, black mangroves had the smallest xylem diameters, the smallest and toughest leaves with the lowest SLA and highest osmolality (Appendix Fig. S1). For seedlings, in contrast, the rank order of species varied by trait, and there were no correlations between adult trait means and that of their offspring (all $P > 0.05$; Appendix Fig. S2).

While seedling trait means did not vary with latitude ($P > 0.2$), the majority of adult traits changed significantly with latitude (Appendix Table S1; Fig. 3). For all species, leaves were smaller, drier, denser and tougher towards the north. In addition, a hierarchical clustering of species’ traits showed two distinct groups. The four northernmost red and three northernmost white populations clustered with all of the black mangrove populations (Fig. 4a), whereas the southern red and white mangrove populations formed a second distinct group that further split into reds versus whites (Fig. 4b).

To examine the relationship between trait convergence and freeze tolerance, we collapsed the adult trait data (except photoinactivation) into principal components. The first principal component (PC1) explained 45.7% of the variation, with increasing values of PC1 linked primarily to increased water content and specific leaf area, and decreased toughness. PC1 declined with latitude for all species (Fig. 3; blacks: $F_{1,85} = 14.8$, $P < 0.001$, $R^2 = 0.12$; reds: $F_{1,69} = 27.7$, $P < 0.0001$, $R^2 = 0.27$; whites: $F_{1,60} = 25.1$, $P < 0.0001$, $R^2 = 0.30$). In addition, declining PC1 values were significantly associated with reduced susceptibility to photoinactivation in red mangroves (red: $t_{1,69} = 2.0$, $P = 0.041$, $\beta = 0.054$). Black and white mangroves showed the same trends although not significantly (black: $t_{1,85} = 1.4$, $P = 0.153$, $\beta = 0.023$; white: $t_{1,60} = 0.1$, $P = 0.914$, $\beta = 0.002$). The second principal components (PC2) explained 14.8% of the variation with increasing values corresponding primarily to decreasing osmolality. PC2 increased with latitude for white mangroves only (Fig. 3; black: $F_{1,85} = 1.1$, $P = 0.283$, $R^2 = 0.04$; red: $F_{1,60} < 0.1$, $P = 0.916$, $R^2 = 0.02$; white: $F_{1,59} = 6.5$, $P = 0.012$, $R^2 = 0.16$) and was uncorrelated with
than in southern populations, suggesting that conditions and seedling growth were higher in range edge populations. In addition, black mangrove freeze resistance produced the most biomass in our common garden experiment. In addition, black mangrove freeze resistance and seedlings were also unaffected by the freeze treatment and produced the least amount of damage after a freeze. Black mangrove seedlings were the most susceptible to freeze damage, their seedlings were the most likely to die after the freeze treatment, and they produced the least biomass. In addition, they showed an increased susceptibility to freezing at the range edge. White mangroves thus appear to be the most easily suppressed by occasional freeze events, coinciding with their limited northern distribution relative to other mangrove species. The northern limit of black and white mangroves already differs by 44.4 km (Williams et al. 2014), and our data suggests that these differences will increase through time.

**Discussion**

To explore why co-occurring species differ in their response to climate change and to elucidate the mechanisms setting range limits, we examined trait variation in three mangrove species along a latitudinal cline in temperature. We found that variation in freeze tolerance, measured by changes in photosynthesis and seedling mortality after a controlled freeze event, paralleled the poleward distributions and expansion rates of three mangrove species. Black mangroves, the most northerly and fastest migrating mangrove species, showed the greatest freeze tolerance, followed by red, and then white mangroves, the least northerly and most slowly migrating species. However, freeze tolerance as well as other ecophysiological traits were not fixed within species, but instead varied systematically with latitude. Adults from black and red mangroves showed higher freeze tolerance towards the range edge, and all three species showed phenotypic convergence on leaves that were denser, tougher and smaller than those in the south. These patterns were consistent across species even though they belong to three separate plant families and showed strong species-level variation in trait means.

If species have historically co-occurred, one might assume that they will respond similarly to shifting environmental conditions. However, our trait-based focus allows us to make stronger predictions than those based on climate envelopes about how different species will respond to climate change. We hypothesize that black mangroves will have the most rapid range expansion, because leaves from both black mangrove adults and seedlings showed the least amount of damage after a freeze. Black mangrove seedlings were also unaffected by the freeze treatment and produced the most biomass in our common garden experiment. In addition, black mangrove freeze resistance and seedling growth were higher in range edge populations than in southern populations, suggesting that conditions at the margin are triggering trait adjustments that could accelerate range expansion. In contrast, we hypothesize that white mangroves will continue to show the slowest range expansion. Their leaves were the most susceptible to freeze damage, their seedlings were the most likely to die after the freeze treatment, and they produced the least biomass. In addition, they showed an increased susceptibility to freezing at the range edge. White mangroves thus appear to be the most easily suppressed by occasional freeze events, coinciding with their limited northern distribution relative to other mangrove species. The northern limit of black and white mangroves already differs by 44.4 km (Williams et al. 2014), and our data suggests that these differences will increase through time.

**Discussion**

Although trait-based analyses often use a mean trait value for each species, trait variation within a species may be important for explaining ecological patterns. We observed that all three species showed a convergence at the range edge towards trait means similar to those of black mangrove. We cannot definitively say that these trait shifts are in response to cold conditions at the range edge because many environmental factors covary with latitude (Lovelock et al. 2007) and these traits serve multiple functions, but our results are not incompatible with the idea that gradients in temperature are driving the latitudinal clines in traits. Toughness is known to affect leaf palatability to herbivores, indicates leaf structural investment, and tends to decrease in shade or with fertilizer (Coley 1983; Hemmi & Jormalainen 2002). Osmolarity is also a measure of drought tolerance (Bartlett et al. 2012), and SLA responds to changes in competition and nutrients (Cook-Patton & Agrawal 2011). However, trait means at the edge converged towards black mangrove means, the most freeze-tolerant species (Figs 3 and 4). We also observed a significant positive relationship between PC1 and photoinactivation in red mangroves (and the same general pattern in black and white mangroves), indicating that declining water content/SLA and increased toughness enabled some measure of freeze tolerance. Finally, most of the trait changes are consistent with observed relationships between these traits and freeze tolerance in other studies.

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**Fig. 4.** Hierarchical clustering of mangrove populations by mean trait values. Tips of the dendrogram indicate individual populations of black mangroves (black lines), red mangroves (red lines) and white mangroves (white lines). Diagonal lines connect populations in the hierarchical clustering to their actual geographic location. (a) All black mangroves populations are clustered together in the upper portion of the dendrogram with the most northern red and white populations. (b) Southern populations of red and white mangroves form a second distinct cluster that is further subdivided by species.

Photoinactivation (black: $t_{1,36} = -0.6$, $P = 0.539$; red: $t_{1,69} = 1.1$, $P = 0.273$; white: $t_{1,60} = 0.5$, $P = 0.602$).

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Leaf size across species declines with altitude (Buot & Okitsu 1999), for example, and others have observed adaptive evolution within species towards smaller leaves and reduced SLA in response to cold stress (Carlson, Holsinger & Prunier 2010). Larger xylem diameters are linked to higher risk of freeze-induced embolisms (Cavender-Bares et al. 2005; Stuart et al. 2006), and increasing osmolality can reduce the risk of freeze damage in both plants and animals (Scarth & Levitt 1937; Kostal et al. 2011). Thus, we speculate that the latitudinal trait shifts we documented may be in response to freezing temperatures, although a covarying trait syndrome rather than a single key trait appears to drive most responses.

Latitudinal gradients in traits could emerge due to trait evolution or phenotypic plasticity. We hypothesize that plasticity is more likely for several reasons. First, trait evolution may be constrained by multiple factors. Mangrove populations are very small at the range edge, which can limit adaptation even in the face of strong selection (Bridle & Vines 2007). Mangrove propagules can also be dispersed broadly via ocean waters and remain viable after long periods of flotation (Triest 2008; Albrecht et al. 2013), which may allow maladaptive gene flow from centre populations (Bridle & Vines 2007). Additionally, mangroves already tolerate highly stressful conditions, including high salinity and anaerobic soils, which may impose strong selective pressures (Etterson 2001) and dampen the ability of mangroves to evolve in response to other selective pressures such as freeze stress. We also observed no significant correlations between adult and offspring trait means, possibly suggesting low trait heritability (Appendix Figure S2), although lack of correlation may be due to differences in developmental stage. We propose instead that trait convergence is due to plasticity, in part because others have observed plasticity in these traits in response to other environmental factors (Simpson, Feller & Chapman 2013) and because we observed latitudinal trends among field-collected samples rather than among individuals reared in a common garden.

A promising follow-up experiment would be to grow northern and southern individuals at both the range centre and edge to definitively test the plastic potential of these traits. If all individuals reared at the range edge show greater freeze tolerance than those reared towards the centre, regardless of their population of origin, then latitudinal trends in freeze tolerance is likely due to plasticity. However, if individuals sourced from range edge populations consistently show greater freeze tolerance than those from more southern populations, then greater freeze tolerance at the range edge is likely due to trait evolution. Moreover, tracking these individuals through time would test whether freeze tolerance overall (or plasticity in freeze tolerance) develops as the trees mature.

In conclusion, our results highlight the importance of taking a trait-based approach to study climate change-induced range shifts. Even though these species co-occur throughout much of their range (Spalding, Kainuma and Collins 2010) and we might expect similar range shifts, a closer analysis of ecophysiological traits illuminates why these species are shifting northwards at different rates. Surprisingly, these species-level differences persisted across the latitudinal cline even though traits consistently converged on a similar freeze-tolerant phenotype. Thus, resistance to freezing may be the predominant driver of mangrove distributions at the edge of their ranges, and reductions in the number of extreme cold events due to climate change are likely to have strong impacts on mangrove communities around the world.

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Data accessibility
All data are included in the manuscript and supporting information.

References