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Cold Comfort: Diversification and Adaptive Evolution across Latitudinal Gradients

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Cold Comfort
Diversification and Adaptive Evolution across Latitudinal Gradients

By

Stephanie Alexandra Stuart

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Committee in charge:

Professor David D. Ackerly, Chair
Professor Todd E. Dawson
Professor Chelsea D. Specht

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Abstract

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Angiosperms originated during a prolonged climatic greenhouse, and their early fossil record comes exclusively from low paleolatitudes. Thus, the ancestral ecological niche of flowering plants was most likely tropical. Tropical origins have shaped the subsequent ecological boundaries and evolutionary opportunities faced by descendents of these ancestors. This has had profound consequences for the subsequent diversification and ecology of this large and important group. Here, these consequences are explored from three different points of view, and at three different scales, with the goal of understanding the evolution of freezing-tolerant clades and the traits that facilitate their survival.

Chapter 1 begins with a broad view of angiosperm evolution, encompassing the entire clade at a global scale. It uses phylogenetically independent contrasts to test the relative contributions of area, latitude, and climate to diversification patterns through time. The analysis shows that expansions in latitudinal range, rather than expansions in total area, are the strongest correlate of increased diversification through the history of this clade. Phylogenetically independent results are then compared with present-day patterns. The present-day latitudinal diversity gradient is demonstrated to be the result of a tropical origin rather than intrinsically higher speciation rates in the tropics.

The origin of the pattern seen in Chapter 1 is explored in Chapter 2, by examining whether greater functional diversity occurs in wet tropical environments, which are the putative ancestral niche of flowering plants, or through adaptation to seasonal environments. Three different rainforest sites in Australia are studied. A wet tropical community is contrasted with two seasonal communities: one which is seasonally dry, and another which is seasonally cold. A link between seasonality and increased functional diversity is demonstrated for traits relating to water use and cold tolerance. A new method is presented for testing the relative contribution of phylogenetic niche conservatism to shifts in trait means between communities. This method is used to demonstrate that, in these three forests, traits relating to successional status are conserved, while traits relating to water used and cold tolerance are evolutionarily labile.

In Chapter 3, a specific hypothesis about the origins of cold tolerance is presented. It is argued that seasonally dry environments could provide an evolutionary stepping stone between wet tropical and

temperate environments, based on a known link between molecular mechanisms of drought and freezing acclimation. Individuals from seven eudicot clades are collected from the same system of wet tropical, dry tropical, and temperate forests used in Chapter 2. On being subjected to a controlled freezing profile, plants from the dry tropical forest show considerably more resistance to damage than their relatives from the wet tropical environment. This demonstrates that acclimation to drought is a plausible pathway for the evolution of tolerance to freezing.

The latitudinal gradient from high species diversity in the tropics to lower diversity at near the poles is often attributed to the intrinsically stressful nature of growing in a seasonal environment. The work presented here refutes this point of view, showing how stress from one perspective can be seen as selective pressure from another. The selective pressures that resulted from transitions into temperate environments in angiosperms have led to more species, increased functional diversity, and greater resistance to unexpected conditions.

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"Sweet changing seasons! …
…Thou givest birth
To shifting scenes of beauty, which outshine
Th' unvarying splendours of the Tropic's clime"

- Alfred R. Wallace, *Tropical Nature*

I saw you standing all alone in the electrostatic rain
I thought at last I'd found a situation you can't explain
With GPS you know it's all just a matter of degrees
Your happiness won't find you underneath that canopy of trees

If the green grass is six and the soybeans are seven
The June-bugs are eight, the weeds and thistles are eleven
And if the ones just hold their place the zeros make a smiley face
When they come floating down from the heavens

-Andrew Bird, *Masterfade*

Introduction

Imagine the Late Jurassic or Early Cretaceous: a warm world, with comparatively little temperature difference between the equator and the poles (Spicer & Chapman 1990; Barron *et al.* 1995; Huber, Hodell, & Hamilton 1995; Herman & Spicer 1996; Retallack 2001; Rees *et al.* 2004). At low paleolatitudes, the arid tropics of the Jurassic were giving way to the warmer and wetter conditions that would characterize the Cretaceous (Barron *et al.* 1995; Rees, Ziegler, & Valdes 2001). Plant communities were dominated by Bennettitalians, conifers, and ferns (Niklas, Tiffney, & Knoll 1983; Lidgard & Crane 1988; Lupia, Crane, & Lidgard 2000; Rees, Ziegler, & Valdes 2001). Such forests had few vines or herbs, and most likely, even few epiphytic ferns (Schneider *et al.* 2004). But, somewhere between 0–30° paleolatitude (Hickey & Doyle 1977; Gübeli, Hochuli, & Wildi 1984; Crane & Lidgard 1989; Brenner 1996; Lupia, Crane, & Lidgard 2000; Barrett & Willis 2001), perhaps near the base of an overturned *Williamsonia*, a scrubby, scandent something scrambles out of the exposed earth. As best we know, this was the origin of angiosperms: tropical, ancient, shaded, and humble (Tiffney 1984; Feild, Arens, & Dawson 2003; Mathews, Burleigh, & Donoghue 2003; Feild *et al.* 2004).

Although the very early origin of angiosperms remains shrouded in mystery, a tropical rooting for the angiosperm phylogeny is now virtually incontrovertible (Chase *et al.* 1993; Mathews & Donoghue 1999; Qiu *et al.* 1999, 2005; Bremer *et al.* 2009; Soltis *et al.* 2011). Current paleobotanical hypotheses also favor a tropical origin for angiosperms, based on the earliest occurrences of pollen and macrofossils (Hickey & Doyle 1977; Gübeli, Hochuli, & Wildi 1984; Crane & Lidgard 1989; Brenner 1996; Lupia, Crane, & Lidgard 2000; Barrett & Willis 2001). Both the ecology of extant basal angiosperms, which are tropical or warm-temperate, and the repeated nesting of temperate angiosperm clades within tropical grades, indicate that this is the ancestral condition for the group as whole (Judd, Sanders, & Donoghue 1994; Feild, Arens, & Dawson 2003; Feild *et al.* 2004).

Today, flowering plants not only cover the globe, but are found in some of the world's most extreme environments—frontiers neither ferns nor gymnosperms have overcome. Angiosperms' incredible diversity of form has allowed them to thrive in environments where ferns make at best tentative advances, and gymnosperms fear to tread. From aquatic to oceanic, arctic to arid, gigantic to tiny, draped over or rooted inside other plants, angiosperms have more growth habits in more environments than any other group of plants—and top it all off with an astonishing number of species (Eriksson & Bremer 1992; Sanderson & Donoghue 1994; Magallón & Sanderson 2001; Davies *et al.* 2004a; Crepet & Niklas 2009).

How did angiosperms get from their humble, tropical beginnings to the situation of virtual world domination we see today? Angiosperms must have faced challenges in adapting to temperate environments. Freezing is recognized as a major stress for many plant groups (Levitt 1980; Sakai & Larcher 1987; Woodward 1987; Pearce 2001). As sessile, exothermic organisms, plants are particularly vulnerable to the seasons: they must find ways to tolerate, resist, or avoid cold (Levitt 1980; Sakai & Larcher 1987; Thomashow 1990). The geographical ranges of both species and vegetation types often directly reflect climatic conditions (Kottek *et al.* 2006; Peel, Finlayson, & McMahon 2007). Yet, the nesting of temperate clades within paraphyletic tropical grades is evidence that transitions between

these two environments have taken place many times, either through vicariance or dispersal (Judd, Sanders, & Donoghue 1994; Wiens & Donoghue 2004; Jablonski, Roy, & Valentine 2006; Smith & Donoghue 2008). These repeated shifts from tropical to temperate are inextricably entangled with the history of global climatic changes. Together, these shape the patterns of angiosperm function and distribution we see today. They may even have played an important role in shaping the dramatically high rates of diversification that characterize this clade (Magallón & Sanderson 2001; Davies *et al.* 2004a; Crepet & Niklas 2009).

This dissertation explores three different aspects of how tropical to temperate shifts have shaped angiosperm evolutionary history. First, I look at how tropical to temperate transitions contributed to the very high diversification rates observed in flowering plants. Second, I compare functional diversity in a wet tropical community with two different seasonal environments. Finally, I explore a pathway that may have made the transition from tropical to temperate possible. Let us begin by setting the stage—what conditions have angiosperms faced through their evolutionary history, and how does this provide the context for the evolution of temperate-adapted angiosperm groups?

Earth's history and the origin of modern-day temperate environments

Climate and carbon models, as well as isotopic records and paleoenvironmental proxies, concur that warm climates, minimal glaciation, and high CO_2 prevailed from the beginning of the Triassic (~251 Ma) to the end of the Eocene (~33.7 Ma, Crowley & Kim 1995; McElwain 1998; Berner & Kothavala 2001; Crowley 2001; Retallack 2001; Royer *et al.* 2004; Royer, Berner, & Park 2007). Angiosperm fossils appeared near the middle of this period (~135 Ma, Hickey & Doyle 1977; Gübeli, Hochuli, & Wildi 1984; Crane & Lidgard 1989; Brenner 1996; Lupia, Crane, & Lidgard 2000; Barrett & Willis 2001). Second-order details of these climates, particularly the difference between equatorial and polar climates, known as the latitudinal temperature gradient, remain an area of active research (Crowley & Zachos 2001; Huber & Caballero 2011). In spite of prevailing warm conditions, the earth may have been cooler during the earliest period of inferred angiosperm evolution—there is evidence for a temperate biome at high latitudes during the Late Jurassic and Early Cretaceous, with tree rings indicating seasonality (Rees, Ziegler, & Valdes 2001).

There appear to be two peaks in CO_2 and temperature during the Cretaceous, one in the mid-Cretaceous near the Cenomanian-Turonian boundary (~91.5 Ma, Huber, Hodell, & Hamilton 1995; Huber, Norris, & MacLeod 2002; Forster *et al.* 2007) and one in the Late Cretaceous, during the Maastrichtian (~70 Ma, Clarke & Jenkyns 1999; Crowley & Zachos 2001; Jenkyns 2003). Crowley and Kim (1995) reconstruct average global temperatures 7.5–8.5°C warmer than the present during the Cenomanian, and CO_2 is believed to have been as much as five times modern preindustrial levels during the Maastrichtian (Crowley 2001). Both periods are associated with very shallow differences between polar and tropical temperatures (Crowley & Zachos 2001).

A consensus also exists that the Paleocene and Eocene were warmer than the present, with high levels of CO_2 (up to two times preindustrial levels) and minimal polar ice (Stott *et al.* 1990; Zachos, Stott, & Lohmann 1994; Miller *et al.* 2005). Though there has also been debate about seasonal gradients during the Paleocene and Eocene (Sloan & Barron 1990, 1992; Sloan 1994), climate estimates based on past

floral and faunal assemblages have consistently pointed to the absence of cold winter temperatures even at high latitudes (Wolfe 1995, 1971; Hutchison 1982, 1992; Spicer & Chapman 1990; Wing & Greenwood 1993; Markwick 1994, 1998; Greenwood & Wing 1995; Sluijs *et al.* 2009; Archibald *et al.* 2010). Results from global climate models now tend to agree (Huber & Caballero 2011).

A dramatic change took place at the Eocene-Oligocene boundary (~33.7 Ma). Although the later Eocene was characterized by a gradual cooling trend of ~7°C over 17 million years, the transition to the Oligocene is marked by cooling so rapid it can only be considered sudden in geologic terms (Zachos *et al.* 2001). Deep-sea temperatures dropped 3–4°C in only ~300,000 years, and ice sheets formed in Antarctica for the first time since the Permian (Crowley & Kim 1995; Zachos, Quinn, & Salamy 1996; Crowley 2001; Liu *et al.* 2009). The change is reflected in floral (e.g., Wolfe 1971; Wing 1987; Retallack 1992; Prothero 1994; Barreda & Palazzesi 2007) and faunal turnover around the world (e.g., Hutchison 1982, 1992; Evanoff, Prothero, & Lander 1992; Zanazzi *et al.* 2007). This was a major change for plants across the globe, most of which had never experienced freezing temperatures (Greenwood & Wing 1995).

This was the beginning of what paleoclimatologists term an extended 'deterioration' in climate—that is, a long-term cooling trend (Zachos *et al.* 2001). The result was a transition towards the ice-house world that has predominated through most of human evolution and is familiar to us today: one with cold seasons, steep latitudinal temperature gradients, and a well-developed cryosphere. Though much of the Oligocene and Miocene (23.8–5.2 Ma) were warmer than the present, from the end of the Eocene, the world would never again be as warm or equable as it had been through the late Cretaceous and Early Tertiary (Stott *et al.* 1990; Zachos, Stott, & Lohmann 1994; Zachos *et al.* 2001). This trend culminated with the ice ages of the Pleistocene (2.588–0.012 Ma), which forced many plants in to cycles of refuge and expansion (Comes & Kadereit 1998; Hewitt 2000; Willis & Niklas 2004). Although there is some evidence that these cycles led to extinctions (Jordan 1992; Jackson & Weng 1999), they are more notable for the occurrence of non-analog vegetation types—communities of extant (or closely related) taxa found in novel combinations under past climates (Jackson & Overpeck 2000; Williams & Jackson 2007).

Flowering plant evolution in the context of past climates

How was the evolution of flowering plants shaped by these trends in global climate? The first unequivocal flowering plant fossils appear in at the beginning of the Cretaceous (Valanginian, ~135 Ma; Hickey & Doyle 1977; Gübeli, Hochuli, & Wildi 1984; Crane & Lidgard 1989; Brenner 1996; Lupia, Crane, & Lidgard 2000; Barrett & Willis 2001), though the stem linage of angiosperms may be much older (Axelrod 1952; Sanderson *et al.* 2004; Bell, Soltis, & Soltis 2005; Magallón & Sanderson 2005; Taylor, Taylor, & Axtell 2008; Soltis *et al.* 2011). Pollen records show angiosperms were initially restricted to low latitudes (Gübeli, Hochuli, & Wildi 1984; Brenner 1996), but spread to mid-latitudes by the Hauterivian (~132 Ma, Hughes & McDougall 1987, 1994; Li & Liu 1994) and were widespread, though not ecologically dominant, by the Barremian (~127 Ma, Barrett & Willis 2001, and references therein). The mid-latitude Jehol biota from China dates to ~125 Ma, and contains whole-plant angiosperm fossils as an ecologically minor component (Zhou, Barrett, & Hilton 2003). Charcolified

flowers have also been found from the Barremian-Aptian (~127–112 Ma) of Portugal (Friis, Pedersen, & Crane 1999).

Combined fossil and molecular dates give the approximate appearance of many major angiosperm groups (Wikström, Savolainen, & Chase 2001; Magallón & Sanderson 2005; Magallón & Castillo 2009; Bell, Soltis, & Soltis 2010). Some of the earliest leaf, fruit and flower fossils are attributed to Magnoliid lineages, which appear between 121–112 Ma (Willis & McElwain 2002, and sources therein). There is also evidence of fruit with affinities to Amborellaceae from this period (Friis, Pedersen, & Crane 2000). Calibrated phylogenies date the divergence leading to eudicots between 156 and 136 Ma; the earliest eudicot fossils appear in the Albian (~112–99 Ma) and are assigned to Platanaceae (Friis, Crane, & Pedersen 1988) and Buxaceae (Wing & Boucher 1998). The earliest fossil monocots—palms—appears in the same period (Wing & Boucher 1998; Willis & McElwain 2002). Rosids and asterids most likely diverged between 180–117 and 112–102 respectively (as Rosiidae and Asteriidae, Wikström, Savolainen, & Chase 2001; Bell, Soltis, & Soltis 2010). Fossil evidence for these lineages shows that members of crown groups within both became widespread between the Turonian (~93–89 Ma) and Maastrichtian (~71–65 Ma, Wing & Boucher 1998; Willis & McElwain 2002). There is also evidence that Malpighiales began to diversify over 100 million years ago (Davis *et al.* 2004).

As a result of this remarkable radiation, Wing and Boucher (1998) suggest that all angiosperm families may eventually be revealed to have originated during the Cretaceous. While this does not seem to be the case for Asteraceae (Kim, Choi, & Jansen 2005; Funk *et al.* 2009; Torices 2010), it is supported for many large, diverse angiosperm clades. The emerging perspective is that major angiosperm clades diverged during a warm Cretaceous, and continued to diversify during a warm and equable Eocene. Willis and McElwain (2002) note that most fossil taxa appearing between 93–65 Ma are closely related to present-day tropical and subtropical lineages. They further argue that increasing warmth during the Cretaceous may have contributed to the rise of angiosperms. Interestingly, through the Maastrichtian (~70 Ma), angiosperm pollen is dominant at lower latitudes, where it makes up 60–80% of palynofloras, but accounts for only 30–50% of higher latitude assemblages (Crane 1987). Angiosperms also show evidence of dramatically increased water-use efficiency, which may be ecologically beneficial at higher temperatures (Boyce *et al.* 2009; Boyce & Lee 2010; Feild *et al.* 2011).

Subsequent changes in climate—especially the development of drier, cooler climates over the past 33 Ma—have undoubtedly contributed to diversification, particularly in Asteraceae and Poaceae (Janis 1993; Jacobs, Kingston, & Jacobs 1999; Kellogg 2001; Kim, Choi, & Jansen 2005; Funk *et al.* 2009; Torices 2010). Nonetheless, there is clear evidence that most of the large and familiar groups of angiosperms had appeared by the end of the Eocene (Magallón, Crane, & Herendeen 1999; Wikström, Savolainen, & Chase 2001; Crepet, Nixon, & Gandolfo 2004; Bell, Soltis, & Soltis 2010). These lineages had enjoyed an equable world for at least 200 Ma, and would have faced cold and freezing conditions for the first time in their evolutionary history at the Eocene-Oligocene boundary (~33.7 Ma). From that point to the present day, conditions at high latitudes have been consistently seasonally cold. The history of how these groups adapted to the cold conditions that would develop over the next 33 million years is the history of our modern flora.

The evolutionary history of tolerance to cold

Today, freezing plays a crucial role in delimiting the habitats in which both crops and wild plants can grow (Levitt 1980; Sakai & Larcher 1987; Pearce 2001), and range limits imposed by freezing are reflected in the long-established and widely recognized floristic differences between temperate and tropical ecosystems (Woodward 1987). Without the ability to seek shelter or regulate body temperature, plants must either be excluded from areas where freezing occurs or develop mechanisms of cold tolerance to survive. Understanding how adaptive traits confer freezing resistance is therefore of both agricultural and ecological interest. Repeated invasions of higher latitudes indicate there are sets of traits that enable survival and competitive success in a temperate environment (Judd, Sanders, & Donoghue 1994; Wiens & Donoghue 2004; Jablonski, Roy, & Valentine 2006; Smith & Donoghue 2008). While many of the mechanisms of freezing damage are well-described, the evolutionary history of adaptation to cold is not as well understood.

In this dissertation, I consider adaptation to temperate environments at three different scales. Chapter 1 is an overview of angiosperm evolution—it explores how the diversity of the clade as a whole has been shaped by its origin in the tropics, and the results of its evolution both within and outside this niche. Chapter 2 is a more focused look at a specific comparison between two tropical communities—one aseasonal, one seasonal—and a temperate community. Finally, Chapter 3 is a mechanistic exploration of whether a specific type of tropical seasonality—dry seasons—could have provided an adaptive pathway to cold tolerance. The goal of this project is to understand the evolution of freezing-tolerant clades and the traits that facilitate their survival. In the process, I will explore not only how these adaptations took place, but also how they shaped the functional and evolutionary diversity of the group as a whole.

Chapter 1: Latitudinal span is a stronger predictor of species richness than area: Variety is the spice of life

Introduction

Latitudinal diversity gradients are one of the oldest and best-known patterns in biodiversity (Hawkins 2001; Willig, Kaufman, & Stevens 2003; Mittelbach *et al.* 2007). Naturalists from the 19th century onward noted the luxuriant diversity of the tropics, and the comparative poverty of temperate regions (e.g., von Humboldt 1850; Wallace 1878; Dobzhansky 1950). The pattern of a tropical peak in species richness is not true for all taxa, but it is a pronounced pattern in many (Willig, Kaufman, & Stevens 2003; Hillebrand 2004), particularly flowering plants (Davies *et al.* 2004b). Flowering plants make a useful system for exploring these patterns because, as primary producers for terrestrial ecosystems, they contribute to diversity gradients in other forms of life as well. Flowering plants are closely tied to the climates in which they grow—unlike animals, they cannot migrate or seek shelter to avoid unfavorable seasons, and are directly dependent on gradients in sunlight and rainfall.

Many hypotheses (over 120 by one count! Palmer 1994) have been proposed to explain patterns of greater diversity the tropics and lower diversity in temperate regions (Mittelbach *et al.* 2007). Most of these hypotheses are not mutually exclusive, and it seems likely that multiple mechanisms have acted, and continue to act, to produce existing gradients. Four major categories of explanations proposed for this pattern are: 1) Climate: the tropics are warmer and get more sunlight, therefore they support more species; 2) Area: the tropics are larger than other biomes, therefore they accommodate more species; 3) Time: greenhouse climates prevailed during the first ~100 Ma of angiosperm evolution (Crowley 2001; Zachos *et al.* 2001; Willis & McElwain 2002), so tropical biomes have existed for much more of angiosperm history, allowing them to accumulate more species; (4) Latitude and phylogenetic niche conservatism: angiosperms are tropical in origin, so as they diversified, they faced fewer barriers to speciation within their ancestral habitat. Inverse hypotheses form a fifth category. These suggest higher diversification outside the tropics, due to the tropical origin of angiosperms, and the relatively recent origin of cooler climates. Under this scenario, angiosperms radiated into emerging temperate areas during cooling periods in the Tertiary and Quaternary (Brochmann & Brysting 2008).

Climate

Early explanations for the latitudinal diversity gradient often focused on the pronounced differences in climate between the temperate and tropical zones (von Humboldt 1850; Hawkins 2001). The greater warmth, higher average insolation, and higher annual precipitation found in the tropics seemed reason enough that plants, in particular, might flourish there. Many ecological theories about tropical diversity posit higher carrying capacities in the tropics (Connell & Orias 1964), implicitly relying on this assumption. According to this hypothesis, diversity toward the poles is limited by abiotic factors (such as freezing or seasonality) and the predominant selective pressure is for stress tolerance (Wallace 1878; Dobzhansky 1950; Fischer 1960). The lack of such limits in the tropics is

conversely thought to make competition the major selective pressure and spur for diversification. Climate is also proposed to have direct impacts on evolutionary processes: warmth may speed rates of molecular evolution (Wright, Gray, & Gardner 2003; Davies *et al.* 2004b).

Area

Not only do tropical regions currently have a larger land area than temperate regions (Terborgh 1973; Rosenzweig 1995; MacArthur & Wilson 2001), their area has also been larger through the majority of angiosperm evolution (Morley 2000; Fine & Ree 2006). Area is thought to influence the potential for speciation by providing more possibilities for environmental heterogeneity and by increasing the chance of isolation between populations (MacArthur & Wilson 2001). Larger areas are also thought to decrease the probability of extinction by increasing average population size and average species range—as well as the probability of access to refugia (MacArthur & Wilson 2001; Mittelbach *et al.* 2007).

Time

Because evolution is a time-dependent process, an older clade will have more descendants than a younger clade in the absence of other effects. A clade that has been in a certain area longer than another clade, given relatively similar generation times, should have more descendants in that area. As a result, the age of different climates, and the time different lineages have had to diversify, play an important role in hypotheses about latitudinal diversity. On the scale of angiosperm evolution, there is reason to believe the tropics are an older habitat than the temperate zone (Morley 2000), because warm regions previously covered more of earth's surface (Sluijs *et al.* 2006). The current rooting of the angiosperm phylogeny suggests that tropical forests were likely the environment in which angiosperms originated (Mathews & Donoghue 1999; Qiu *et al.* 1999), as does data from plant function (Feild, Arens, & Dawson 2003; Mathews, Burleigh, & Donoghue 2003; Feild *et al.* 2004). There is also evidence that many temperate angiosperm clades are nested within tropical groups, and thus probably descended from tropical ancestors (Judd, Sanders, & Donoghue 1994; Jablonski, Roy, & Valentine 2006; Jansson & Davies 2008).

Latitude and phylogenetic niche conservatism

The putative tropical origin of angiosperms leads to a fourth hypothesis. Theory suggests that phylogenetic niche conservatism may result in stronger barriers to latitudinal dispersal than longitudinal dispersal (Diamond 1997; Wiens & Donoghue 2004). This hypothesis does not propose that diversification rates are higher in the tropics; instead it proposes that there are more plant species in the tropics because angiosperms are historically adapted to live in the tropics. However, it also has a corollary: plants that encounter new conditions in different climates are more likely to become isolated from their parent populations. Studies of niche breadth show specialization is particularly likely to occur in response to stressful conditions (Thuiller *et al.* 2004; Boulangeat *et al.* 2011), and conditions of seasonal cold are expected to be stressful to tropically-adapted plants (although whether niche breadth is wider in tropical or temperate regions is an open question, see below and Janzen 1967; Loehle 2000; Condit *et al.* 2006.) Under this framework, clades that expand their ranges north-to-south are more likely to give rise to new species than those that do not. This is

true in both hemispheres. Although there is greater poleward land mass in the northern hemisphere (excluding Antarctica), both hemispheres are characterized by similar equator-to-pole gradients in seasonality and temperature (Petersen, Sack, & Gabler 2011).

Inverse gradients in diversification: temperate radiations

A related, but distinct hypothesis predicts that when historical effects are controlled, higher speciation rates will actually be found in temperate regions. Climate-type classifications suggest more different climatic categories within temperate regions than within tropical ones (Mu□ller 1982). If differing conditions lead to new species, this could result in higher diversification in temperate environments. Furthermore, if angiosperms are historically predominantly tropical, lineages that adapted to cold would have had access to a region with less competition, and may have been able to radiate in these new areas. As an argument against this hypothesis, it has also been suggested that seasonal conditions provide selective pressure for plants to become generalists, and that, as a result, species ranges and effective population sizes tend to be larger in temperate regions (Janzen 1967). This could potentially lead to lower rates of speciation.

Table 1: Predictions made by the five hypotheses examined in this chapter

	Prediction
Climate	Higher diversification in clades found in warmer, less seasonal climates
Area	Higher diversification in clades with larger ranges
Time	Different significant effects under PIC and Tip frameworks
Latitude	Higher diversification in clades that span many degrees of latitude
Temperate radiations	Higher diversification in clades exposed to cooler, seasonal climates

What this chapter tests

Each of these five hypotheses predicts a distinct evolutionary pattern (Table 1). These patterns can be tested using phylogenetic independent contrasts (PICs; Felsenstein 1985). By looking at differences between sister clades, PICs allow independent analysis of individual evolutionary divergences. Comparing differences in diversity with differences in another aspect of clade biology is a direct test of whether that aspect is linked to higher diversification rates. This phylogenetic framework is combined with spatially explicit mapping of range areas and resulting climate envelopes.

Methods

Phylogeny and focal clade selection

Unlike studies of trait evolution, analyses of diversity must use fairly large monophyletic groups as the units of analysis. Here, angiosperm families are used as a starting point, as circumscription of most is now monophyletic (Stevens 2001; APG II 2003). In addition, family-level hypotheses for phylogenetic relationships in flowering plants are increasingly well understood (Donoghue & Doyle 1989; Chase *et al.* 1993; Nandi, Chase, & Endress 1998; Soltis, Soltis, & Chase 1999; Qiu *et al.* 1999, 2005; Savolainen *et al.* 2000; Davies *et al.* 2004a).

Phylogenies were downloaded from Phylocom (Webb, Ackerly, & Kembel 2008). A list of 408 family-level clades was made by reconciling the families in the Davies *et al.* (2004a) supertree with the maximally resolved tree based on the work of Stevens (2001). The Davies supertree was then used as the phylogenetic hypothesis for the remaining analyses.

Family range mapping

Range maps were drawn from Stevens (2001) and Heywood *et al.* (2007) to create a complete set for the selected family list. Circumscription of families followed Stevens (2001). When the two sources conflicted after accounting for circumscription, the source citing the most recent references was favored. Range estimates for taxa that occurred on Pacific islands posed a special problem, as most global-level maps are too coarse to show plant ranges on these small land masses. Although both Heywood *et al.* (2007) and Stevens (2001) occasionally highlight islands with endemic plant families, an initial pass showed that this underestimated island diversity. To compensate, seven biogeographic regions were identified in the Pacific (after Udvardy 1975, but adding the Galápagos as an additional region.) A list of all families was extracted from at least one flora from within each province: Papuan (Foreman 1971; Borrell 1989), Micronesian (Fosberg & Sachet 1987), Hawaiian (USDA 2008), Southeastern Polynesian (Wester 1985), Central Polynesian (Hotta 1962), New Caledonian (Guillaumin 1948), East Melanesian (Paraham 1972), and the Galápagos (Wiggins & Porter 1971). In all cases, family range maps represent a best-guess of distributions. Actual ranges are certain to be patchy within the areas depicted, due to microclimate, edaphic variation, and altitude. Although the large number of families included should mitigate accidental bias, ranges were not adjusted for any of these the effects.

Family range maps were downloaded at low resolution from Stevens (2001). These maps were projected onto equal-area maps with a 100 × 100 km pixel size. Higher-resolution equal area maps were created by expanding the maps and editing them by hand for pixilation, using Adobe Photoshop (CS2, Adobe Systems Inc., San Jose, CA) with reference to taxonomic sources (Stevens 2001; Heywood *et al.* 2007). Where family-level circumscriptions had changed, range maps were combined or redrawn. ArcGIS was used to produce equal-area range map rasters, which were used for all subsequent calculations in the R statistical environment (R Development Core Team 2011).

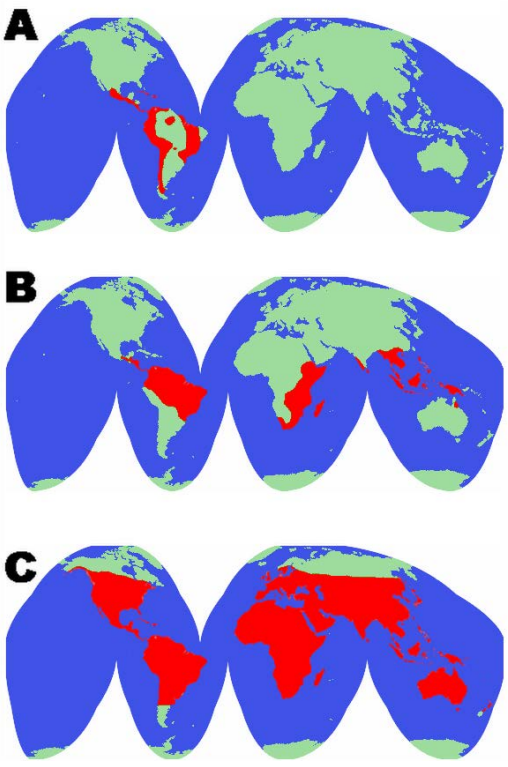


Figure 1: Range types Examples of clades with large latitudinal span (A, Alstroemeriaceae), large longitudinal span (B, Achariaceae), and large total area (C, Amaranthaceae).

Area, latitude, and longitude

The effect of area on diversification rates is undoubtedly conflated with shifts in climate during earth history (Fine 2001; Fine & Ree 2006). This presents a significant challenge for analyzing present-day patterns. Here, I develop a method for considering the evolution of species ranges within a phylogenetic error structure. This framework considers two different aspects of area, longitude and total area. I also consider latitude as a separate category. "Latitude" constitutes the vertical axis of a clade's range, while "longitude" constitutes the horizontal axis. Clades that have

both a large range in latitude and longitude may have a large total area, or a crooked range with a smaller area (Figure 1).

Area was extracted directly from equal-area projections of family ranges. Latitude was obtained by overlaying the family range maps on a latitudinal data grid extracted from ArcGIS. Several types of latitude data were considered, including minimum latitude, maximum latitude, and latitudinal span (maximum latitude - minimum latitude) in each hemisphere, as well as total latitudinal span across both hemispheres. In order to avoid exaggerating the range of each family due to a small projection at an extreme latitude, I also considered the highest latitude reached by the family in 99% of the area of its range, in both hemispheres. Longitude was calculated as the sum of the family's maximum horizontal range in kilometers across an equal-area projection. Kilometers were used rather than degrees as the total length represented by a degree of longitude varies greatly with latitude. Longitude as measured here is the total longitudinal span, not the contiguous longitudinal span, and in some cases represents the sum of longitudinal span on several continents (ocean is not included, however.)

Climate

World-wide climate maps with nineteen measurements of temperature and precipitation from BioCLIM, as provided by the WorldClim dataset (Nix 1986; Busby 1991; Hijmans *et al.* 2005) were imported to R, where they were overlaid with family range maps. BioClim includes 19 variables, with values calculated as listed inTable 2. For each family range, a single value was calculated for each climatic variable. In most cases, this was an average across the geographic range of the family, with each pixel given equal weight. As an example, the value for mean annual temperature would be the average annual temperature of each pixel, averaged across all pixels where the family is found. This is the "geographical average of mean annual temperature." For maximum and minimum temperature, I chose to use the highest and lowest values found in the range, respectively. This are referred to as the "maximum temperature in family range" and the "minimum temperature in family range." Note that physical location at which the minimum temperature occurred may be geographically distant from that of the maximum temperature. Precipitation variables were averaged across all pixels in each family range. This is referred to as "geographical average precipitation." Table 2 gives the method used to calculate each variable, both in BioCLIM at the pixel (temporal) level and in this chapter at the family range (geographic) level, and indicates whether averaging or extreme values were used.

Principal components analysis

Exploratory analysis using BioCLIM data from each clade's geographical range showed many of the variables were strongly non-orthogonal. This resulted in a severe distortion of effect sizes and directionality. To overcome this, I conducted a principal components analysis on the family-level climate values. Separate analyses were used for temperature and precipitation related variables (BioCLIM 1–11 and 12–19 respectively; Table 2). From each principal component analysis, the first three components were selected, accounting for ~92% of variance in temperature data, and ~85% of variance in precipitation (Table 3). The resulting principal component scores for each family were

used directly in the Tip regressions, and used as a basis for calculating PICs for the comparative phylogenetic analyses.

Phylogenetic methods

Phylogenetically Independent Contrasts (PICs) were calculated using the phylogeny of Davies *et al.* (2004a). For climate data, Felsenstein's algorithm (1985) was used, calculating mean values for

Table 2: Variables used in this analysis Explanations of BioCLIM pixel-level calculations from WorldClim

	BioCLIM pixel-level calculations	*Range-wide calculation*
Area		
Latitude		
Longitude		
BC 1 - Mean Annual Temperature (°C)	12 month mean	Mean
BC 2 - Mean Diurnal Temperature Range (°C)	(Mean of monthly (max temp – min temp))	Mean
BC 3 - Average of Isothermality	(Mean diurnal range / annual temp range) × 100	Mean
BC 4 - Temperature Seasonality	(Standard deviation × 100)	Mean
BC 5 - Maximum Temperature (°C)	Maximum	Maximum
BC 6 - Minimum Temperature (°C)	Minimum	Minimum
BC 7 - Annual Temperature Range (°C)	Max temp of warmest month - min temp of coldest month	Mean
BC 8 - Mean Temperature of Wettest Quarter (°C)	3 month mean	Mean
BC 9 - Mean Temperature of Driest Quarter (°C)	3 month mean	Mean
BC 10 - Mean Temperature of Warmest Quarter (°C)	3 month mean	Mean
BC 11 - Mean Temperature of Coldest Quarter (°C)	3 month mean	Mean
BC 12 - Annual Precipitation (mm)	Cumulative	Mean
BC 13 - Precipitation of Wettest Month (mm)	Cumulative	Mean
BC 14 - Precipitation of Driest Month (mm)	Cumulative	Mean
BC 15 - Precipitation Seasonality	Coefficient of variation	Mean
BC 16 - Precipitation of Wettest Quarter (mm)	Cumulative	Mean
BC 17 - Precipitation of Driest Quarter (mm)	Cumulative	Mean
BC 18 - Precipitation of Warmest Quarter (mm)	Cumulative	Mean
BC 19 - Precipitation of Coldest Quarter (mm)	Cumulative	Mean

contrasts at deeper nodes. For species richness contrasts, the algorithm of Agapow and Isaac (2002) in which diversity values are summed at deeper nodes, was used. For area, latitude, and longitude, a new method was developed to calculate PICs for geographically structured data (see below). Contrasts were not standardized to branch lengths because Brownian motion models are not appropriate for diversification or geographical data. All algorithms used a method based on Pagel (1992) to handle polytomies. This conservative method calculates a single contrast for each polytomy, and does not assume any particular underlying resolution. Pagel's algorithm sorts the daughter branches for the first character analyzed from greatest to smallest, then splits on the average value; the same split is followed for subsequent characters, regardless of their values. My method used a single split for all characters, regardless of their values, rather than choosing an initial split based on a single character. The split was based on the computational method used to store the phylogeny.

Phylogenetically independent contrasts for geographic data

Range area is not, strictly speaking, heritable. However, allopatric, parapatric, and sympatric speciation all imply that a descendant species will share, equally or unequally, different parts the parent species' range. Range area may also act as a proxy for traits that either allow a species to disperse across and colonize a large range area, or limit it to primarily local dispersal and persistence. I extended the technique used by Agapow and Issac (2002) to include new approaches for three phylogenetically structured types of data that accumulate through time: geographical range area, latitudinal span and longitude. To calculate PICs of geographical range areas, I started with mapped ranges for each clade. At each node, I took the geographical union of daughter clade areas—meaning that any pixel where either daughter occurred was included in the union that represented the ancestral node, but that pixels where both daughters occurred were not counted twice (Figure 2). I then calculated the area of the union, which was stored as the value associated with that node. PICs were calculated from these stored values by subtraction (Felsenstein 1985; Garland, Harvey, & Ives 1992; Pagel 1992). The same approach was applied to latitudinal span; the latitudinal span for each node was the union of the latitudes of its daughter nodes. Longitudes were recalculated at each node from the union of descendant clade ranges, and contrasts were calculated by subtracting node values. Note that this method results in successively larger values for area, latitude or longitude at deeper nodes, eventually encompassing the entire globe at the root of the tree. Thus, this is not an estimate of actual ancestral areas. Rather it measures the areas occupied in

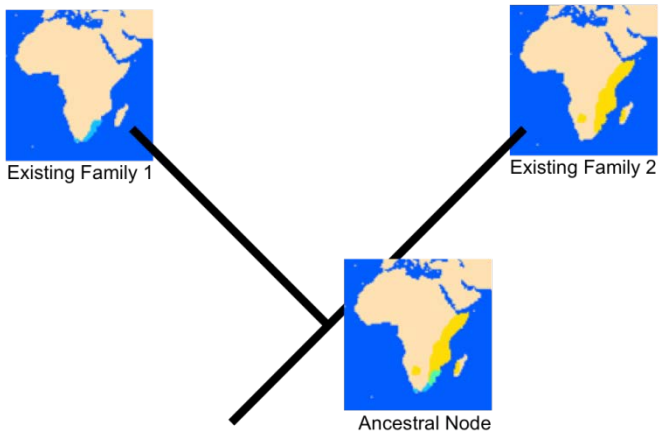


Figure 2: Example of Geographical PICs Method for calculating descendant range for deeper nodes. Blue indicates the range of Existing Family 1; yellow shows the range of Existing Family 2; and light green shows the area where they overlap. The total area associated with the ancestral node shown here would be the area indicated by all three colors. As a result, the green area is not counted twice.

the present by the descendent taxa at each node, as a measure of their opportunity for diversification. There may be other types of data that show phylogenetic structure, but are not appropriately treated through averaging, and might benefit from further modified PIC approaches.

Linear Model Analysis

Linear model analysis was performed in the R statistical environment (R Development Core Team 2011) with the packages car (Fox & Weisberg 2001, 2011), ape (Paradis, Claude, & Strimmer 2004; Paradis 2006; Paradis *et al.* 2011) and picante (Kembel *et al.* 2010, 2011). PIC-based analyses were used to account for phylogenetic error structure and to consider historical patterns of diversification in place of standing diversity. I contrasted the results from these with Tip analyses, based on linear analysis of data for each family only. Both types of analysis were performed in the same model framework, with the exception that PIC analyses were always forced through the intercept (Garland, Harvey, & Ives 1992). All variables were centered at zero and scaled to the standard deviation before analysis. For PICs, which have an expected mean of zero, the standard deviation is calculated using n degrees of freedom, rather than the usual n–1. Type III sums-of-squares (simultaneous calculation for all variables in the analysis, acceptable when interactions are not considered) were used in all subsequent analysis of regression results. This makes the resulting effect sizes independent of the order of addition of the model terms, an important consideration when trying to distinguish which explanatory variable is the best predictor of the response variable. Before combining each set of

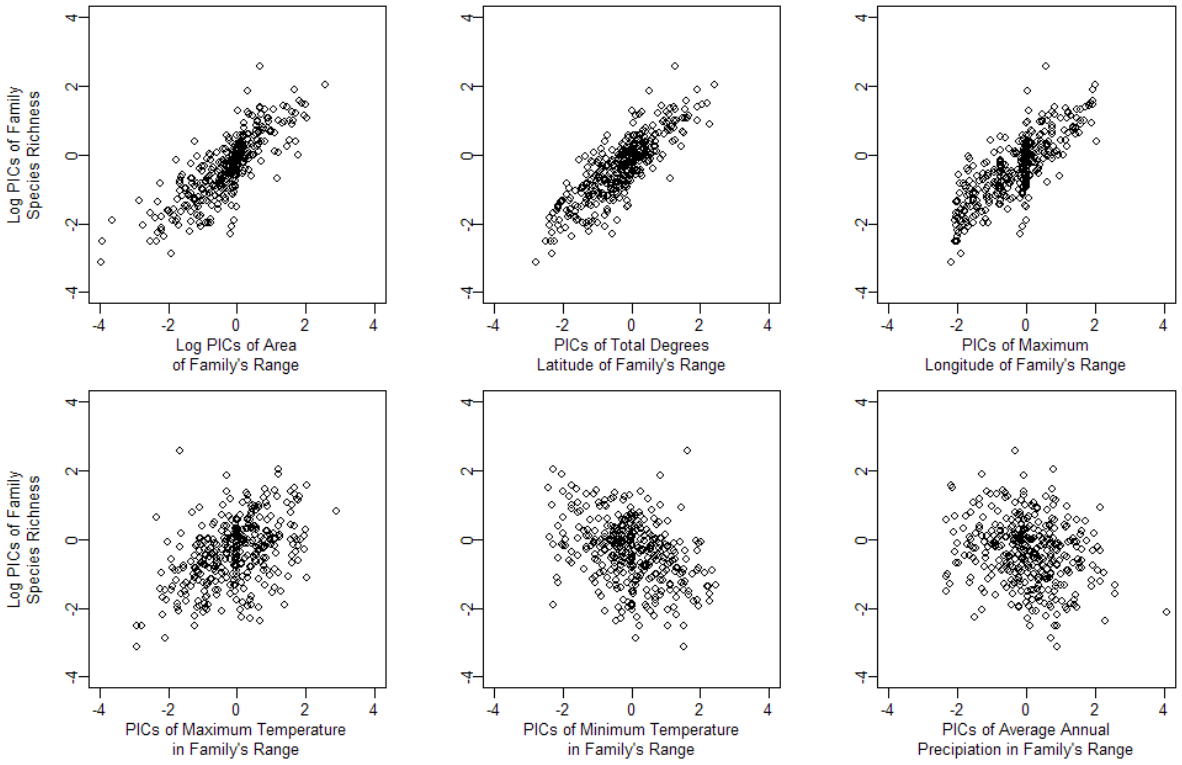


Figure 3: Relationships between independent contrasts in diversity and other variables Bivariate relationships between differences in species diversity and area, latitude, and longitude (top row) and maximum temperature, minimum temperature, and precipitation (bottom row.)

variables in a linear regression model, I tested the orthogonality of the variables using the variance inflation factor (VIF, Chatterjee & Price 1977). Only variable combinations with a VIF under ten were combined in any one model. For PIC, total latitude, area, and longitude, were distinct enough to be combined in a single analysis. However, for Tips, maximal longitudinal span and area were not sufficiently orthogonal to be included in a single model, and were considered separately.

Results

Phylogenetically independent contrasts between species richness and other predictors suggest evolutionary divergences in species richness are correlated with divergences in area, temperature and latitude (Figure 3). This indicates that, in comparisons of sister taxa, an increase in any one of these predictors is linked to an increase in species richness.

Results of the principal components analyses

PCA analysis of the temperature-related BioCLIM variables as measured across angiosperm family ranges found a strong signal of overall warmth and seasonality in the first principal component axis

Table 3: Interpretation of principal components analysis The proportion of variance explained by each component is listed under "Explained." Cumulative variation explained by adding components is listed under "Cumulative." Loadings for each variable on each axis are given in parenthesis after the variable name.

			Temperature			
	Explained	*Cumulative*	*Dominant variables*	*Interpretation*	*A low score suggests*	*A high score suggests*
PC 1	0.695	0.695	Mean of Coldest Quarter (-0.36) Mean Annual Temperature (-0.35) Temperature Seasonality (0.35) Annual Range (0.34) Mean of Driest Quarter (–0.34) Isothermality (0.33) Mean of Wettest Quarter (0.29)	Even loading across several variables, indicating overall warmth with low seasonality	The environment is tropical, with low seasonality, no cold winter, and a high mean annual temperature	The environment is temperate, with low mean annual temperature, a cold winter, and high seasonality
PC 2	0.151	0.846	Maximum (–0.66) Mean Diurnal Range (–0.46) Mean of Warmest Quarter (–0.36)	Extreme daily highs, warm summers	Hot days and cool nights during warm summers, resulting in a large diurnal range	The absence of a warm summer, with smaller daily ranges
PC 3	0.081	0.927	Mean Diurnal Range (–0.81) Minimum (–0.34) Mean of Wettest Quarter (0.33)	Temperature range in a day, especially to cold	A large diurnal range, without low minimums, and a winter wet season	Smaller daily temperature range, low minimums and wet summers
			Precipitation			
	Explained	*Cumulative*	*Dominant variables*	*Interpretation*	*A low score suggests*	*A high score suggests*
PC 1	0.531	0.531	Annual Precipitation (0.47) Wettest Quarter (0.45) Driest Quarter (0.43)	Even loading across longer-timespan variables, indicating total precipitation	The environment is very dry	The environment is very wet
PC 2	0.197	0.729	Seasonality (0.58) Wettest Month (0.52) Driest Month (–0.43)	Precipitation seasonality	Rainfall is not very seasonal, and there is little difference between wet and dry seasons	Rainfall is very seasonal; the dry season, very dry, the wet season very wet
PC 3	0.118	0.847	Driest Month (0.61) Seasonality (0.43) Warmest Quarter (0.34)	Overall aridity	The dry season is very dry, and the environment is not very seasonal	The environment is seasonal, but the driest month is still fairly wet

(Table 3). Because the tropics are less seasonal than temperate regions, most of the variability along this axis is due to the presence or absence of a cold winter. Thus, a high Temp-PC1 score indicates a temperate-type climate, with a low mean annual temperature, and a low score indicates a tropical-type climate with low seasonality, a high mean temperature, and no low minimums.

With the first principal component dominated by a gradient from warm to cold temperatures, the second principal component picks up the effect of warm temperatures (Table 3). A low Temp-PC2 score indicates a climate with high maximum temperatures, a large diurnal temperature range, and hot summers. A high score suggests the opposite: low maximum, low diurnal range, and cool summers. The third principal component describes remaining variation in diurnal temperature range, with a low score indicating large diurnal differences, and a high score indicating small differences. Minimum temperature and the temperature of the wettest quarter also load on this axis.

In the principal component analysis of precipitation, the first principal component axis showed a clear signal of overall precipitation throughout the year (Table 3). This was particularly evident in the even loading of variables integrating a longer time span, such as annual precipitation and all four quarterly precipitation variables. The second principal component axis was strongly related to seasonality of rainfall, with high precipitation values for wet months, and low precipitation values for dry months. The third axis was dominated by aridity, with a high score indicating a highly seasonal environment with a comparatively wet dry season, and a low score indicating a very dry, aseasonal environment, such as some deserts.

Latitude is the best predictor of diversification

Multiple linear regression was used to explore whether climate, area, or latitude was a better predictor of phylogenetic variation in species richness. Differences in total latitudinal span were by far the best predictor of species richness, with a positive association between total latitudinal span and diversity explaining about 10% of variation in species richness (Table 4). Contrasts in maximum longitudinal span were also a significant predictor of differences in species richness, but accounted for only about half as much variation as contrasts in latitude (~5%; Table 4). By contrast, in this order-independent regression, changes in area were only marginally significant ($p < 0.10$), and explained very little of differences in species richness.

Some contrasts in climate also showed a significant relationship with diversity. Differences in Temp-PC1 were positively related to differences in species richness. This indicates clades that scored high on this axis tended to have higher diversity than sister clades with low scores on this axis. A high score on Temp-PC1 indicates a more seasonal environment with a lower mean annual temperature. In this analysis, such an environment is clearly associated with higher species richness, explaining ~3.9% percent of observed variation.

Table 4: Best model, multiple regression using PICs

	Estimate	*Std. Error*	*t value*	*p*	*Type III Sum Sq*	*df*	*F*	*p*	*% variance explained*
Latitude	0.470	0.07	6.69	$8.9e^{-11}$ ***	10.05	1	44.72	$8.8e^{-11}$ ***	10.00%
Longitude	0.328	0.07	4.78	$2.5e^{-6}$ ***	5.15	1	22.89	$2.5e^{-6}$ ***	5.12%
Temp PC1	0.214	0.05	4.15	$4.1e^{-5}$ ***	3.88	1	17.24	$4.1e^{-5}$ ***	3.85%
Area	0.142	0.08	1.86	0.063	0.78	1	3.47	0.063	0.78%
Temp PC2	0.058	0.04	1.48	0.140	0.49	1	2.19	0.140	0.49%
Temp PC2	–0.064	0.05	–1.36	0.174	0.42	1	1.86	0.174	0.42%
Precip PC1	0.048	0.07	0.70	0.486	0.11	1	0.49	0.485	0.11%
Precip PC3	–0.016	0.03	–0.52	0.604	0.06	1	0.27	0.604	0.06%
Temp PC3	–0.020	0.05	–0.42	0.673	0.04	1	0.18	0.673	0.04%
Residuals					79.6	354			
Total					100.5				

Significance: 0 < *** < 0.001 < ** < 0.01 < * < 0.05

Residual standard error: 0.474 on 354 degrees of freedom. Multiple R^2: 0.78, Adjusted R^2: 0.78

$F_{9,354}$: 140.1, $p < 2.2e^{-16}$

Table 5: Comparison of different PIC regression models The effects of excluding area, latitude, longitude, and Temp PC1. Significance values shown are for the Type III Sum of Squares ANOVA model.

	Model 1		*Model 2: – Area*		*Model 3: – Latitude*		*Model 4: – Longitude*		*Model 5: – Temp PC 1*	
	Type III Sum Sq	*variance explained*	*Type III Sum Sq*	*variance explained*	*Type III Sum Sq*	*variance explained*	*Type III Sum Sq*	*variance explained*	*Type III Sum Sq*	*variance explained*
Area	0.781	0.78%	-	-	9.86 ***	8.12%	4.44 ***	3.90%	0.95 *	0.84%
Latitude	10.05 ***	10.00%	19.13 ***	16.90%	-	-	20.02 ***	17.60%	10.69 ***	9.45%
Longitude	5.15 ***	5.12%	8.8 ***	7.76%	15.12 ***	12.45%	-	-	4.36 ***	3.85%
Temp PC1	3.88 ***	3.85%	4.05 ***	3.57%	4.517 ***	3.72%	3.09 ***	2.72%	-	-
Temp PC2	0.42	0.42%	0.52	0.46%	0.439	0.36%	0.28	0.24%	4.02 ***	3.56%
Temp PC3	0.04	0.04%	0.04	0.03%	$1.8e^{-4}$	$1.0e^{-6}$	$1.1e^{-5}$	$1.0e^{-7}$	3.59 ***	3.18%
Precip PC1	0.11	0.11%	0.13	0.11%	0.08	0.07%	0.28	0.24%	5.17 ***	4.57%
Precip PC2	0.49	0.49%	0.25	0.22%	1.81 **	1.49%	0.86	0.76%	0.03	0.02%
Precip PC3	0.06	0.06%	0.16	0.14%	$1.4e^{-4}$	$1.0e^{-6}$	0.04	0.04%	0.85	0.75%
Residuals	79.6		80.3		89.6		84.7		83.4	
Total	100.5		113.4		121.4		113.7		113.1	
Multiple R^2	0.78		0.78		0.75		0.77		0.77	
Adjusted R^2	0.78		0.77		0.75		0.76		0.77	

Significance: 0 < *** < 0.001 < ** < 0.01 < * < 0.05

Table 6: Best multiple regression models using present-day values only (Tips)

	Model 1		*Model 2*	
	Type III Sum Sq	*variance explained*	*Type III Sum Sq*	*variance explained*
Intercept	*ns*	*ns*	*ns*	*ns*
Area	5.99 ***	18.74%	-	-
Latitude	0.86 ***	2.69%	4.54	12.69%
Longitude	-	-	4.22 ***	11.79%
Temp PC1	0.7 ***	2.19%	0.94 ***	2.62%
Temp PC2	0.14	0.44%	$1.2e^{-3}$	0.003%
Temp PC3	0.18	0.56%	0.21	0.59%
Precip PC1	0.01	0.02%	$4.6e^{-5}$	0.0001%
Precip PC2	$3.4e^{-3}$	0.01%	0.03	0.09%
Precip PC3	0	0.00%	$1.1e^{-3}$	0.003%
Residuals	24.08 ***		25.85 ***	
Total	31.96		35.8	
Multiple R^2	0.72		0.7	
Adjusted R^2	0.72		0.69	

Significance: 0 < *** < 0.001 < ** < 0.01 < * < 0.05

Table 5 compares the effects of removing different model terms on the overall explanatory power of the entire model, and demonstrates the degree to which remaining model terms pick up residual variation. Omitting area from the model has very little effect; but omitting latitude decreases both the multiple and adjusted R^2. Omitting Temp-PC1 also has a small, but distinct effect, decreasing the overall explanatory power of the model.

These results can be contrasted with results from an analysis of present-day family diversity and against area, latitude, and climate that does not include evolutionary history (Table 6). This analysis found that area was the best predictor of current diversity, explaining nearly ~19% of variation. A model with area and latitude explained ~25% of the variation in present-day family diversity, while a model with latitude and longitude explained slightly more, ~28%. All three variables could not be included in a single regression because of non-orthogonality. As in the PIC analysis, Temp-PC1 was also a significant predictor of species richness, accounting for a similar, but slightly smaller, 2.2–2.6%.

Discussion

This study set out to test five hypotheses: 1) Tropical climates lead to higher diversification rates; 2) Larger range areas lead to higher diversification rates; 3) Time-for-diversification contributes to currently observed gradients in species richness; 4) Latitudinal span is a better predictor of species richness than area; 5) When time-for-speciation is controlled, higher diversification rates occur in temperate, rather than tropical, climates. The results presented here firmly reject hypotheses one and two, and provide strong support for hypotheses three, four and five.

Temperate, not tropical clades, have higher diversification rates

I did not find any evidence that warmer climates were linked with higher diversification rates. When contrasts in temperature were considered alone, absolute maximum temperature was correlated with higher diversity (Figure 3). Maximum temperature alone, however, cannot separate the effects of overall temperature and seasonality, since both temperate and tropical climates may have high temperatures during part of the year. Instead, if the warmth of tropical environments contributes to higher rates of diversification, the absence of cooler temperatures should be an important predictor of diversification. The opposite was observed: low minimum temperatures were also correlated with high diversity, hinting that ranges encompassing both extremes might actually be the most diverse. This was found to be the case when principal components were used to describe the primary axes of variation in temperature and seasonality. Scores on Temp-PC1 that indicated cold winters and high seasonality were clearly associated with higher diversity in multiple regression models (Table 3). Although Temp-PC1 accounts for a relatively small amount of total variation in species richness contrasts—just 3.9%—it is nonetheless significant. This small but important contribution is what might be expected if the diversification of temperate clades is a relatively recent historical pattern—for instance, one associated with the appearance of cooler, more seasonal climates since the Oligocene (Zachos, Quinn, & Salamy 1996; Pearson, Foster, & Wade 2009).

Latitude is a better predictor of species richness than area

No evidence was found to support the hypothesis that larger areas have contributed to higher speciation rates through evolutionary history. Although area was highly significant in the Tip analysis, it was not significant in the independent contrast analysis. This strongly suggests that the apparent influence of area is the result of historical processes during angiosperm evolution. By contrast, latitude emerged as a much better predictor of differences in species richness than either area or longitude. This suggests that it is not merely large ranges that result in high diversity. Instead, it supports the phylogenetic niche conservatism hypothesis, which suggests that the location and shape of the range is far more important. It also confirms the proposition that north-to-south ranges, which cover many different climates, result in speciation in response to contrasting selective pressures.

Larger longitudinal (east-to-west) ranges appear to make a small but independent contribution to higher rates of diversification. This may be for some of the reasons suggested for area—they increase the chance of isolation between populations (MacArthur & Wilson 2001) or the probability of access to refugia (MacArthur & Wilson 2001; Mittelbach *et al.* 2007). Alternatively, east-to-west ranges often cross gradients from littoral to continental, especially at local scales (e.g., Major 1977). Perhaps, as with latitude, it is not any one climate that is most important, but the exposure to different types of conditions.

Time-for-diversification best accounts for the latitudinal gradient in species richness

As a whole, these results strongly suggest that time-for-diversification, along with the constraints of phylogenetic niche conservatism, are the major contributors to present-day latitudinal diversity gradients. The results of PIC (Tables 4 and 5) and Tip (Table 6) analyses are dramatically different. If only contemporary patterns among my monophyletic groups are considered, area is a good predictor of species richness, explaining nearly 20% of variation in diversity. However, once phylogenetic contrasts are considered, it explains less than 1%, highlighting the importance of differentiating between pattern and process.

It is worth noting that no other aspects of either temperature or precipitation were significant predictors of differences in species richness. Indeed, principal components of the precipitation data were rarely significant, and explained less than 1% of differences in species richness, unless temperature was excluded from the model. This is initially surprising, given the clear effect of climatic patterns on the distribution of vegetation, and the apparent close adaptive fit between most plants and their environments (Mu□ller 1982; Woodward 1987; Ackerly 2003). However, diversity should not be conflated with fitness. Over the timescales examined here, plants have undoubtedly undergone adaptation in response to climatic parameters, including precipitation and aspects of temperature other than seasonality. What this analysis establishes is that differences in these parameters are not associated with higher diversification rates—for instance, there is no evidence that clades in wetter environments have higher rates of speciation than clades in drier environments.

Finally, this analysis was limited to patterns that could be recognized at the level of large clades such as those that underlie most monophyletic families. I recognize that this analysis is a preliminary approach using the best hypotheses currently available, and hope that it will be useful in generating further hypotheses for more detailed testing at lower hierarchal levels.

Conclusions

Contrary to many common-sense hypotheses, greater area and warm climates are not linked to higher rates of speciation in angiosperms. As a result, the dramatic present-day latitudinal diversity gradient in this group appears to be primarily the result of its tropical origin and phylogenetic niche conservatism. This is supported by the finding that, once evolutionary history is considered, greater seasonality is linked to higher speciation rates.

The hypotheses that opened this chapter may be seen as a classic argument between two worldviews: the first, held largely by ecologists, sees the world as tending toward equilibrium, and attempts to define the natural laws that describe such equilibrium. Evolutionary biologists, by contrast, tend to hold the view that all present-day patterns are merely the stochastic result of historical contingency. The result of this chapter is a synthesis: when considering large numbers of clades, it is possible to detect underlying patterns that lead to the same results repeatedly over evolutionary time, resulting in broad-scale patterns. However, it is also important to recognize that these patterns are made up of many accidents and unlikely events. Broad-scale patterns can be seen in the association between expansion of latitudinal range and increase in speciation. This appears to be the result of a predictable truth of natural selection: exposure to different types of selective pressure in different parts of a clade's range will lead to divergence. By contrast, the smaller effect detected, linking greater seasonality with higher speciation rates, is an example of a historical accident: had the relevant adaptive environment of the clades' most recent common ancestor, or the fluctuations in Earth's climate during the evolutionary period been different, this most likely would not have been observed.

In conclusion: tropical floras are diverse because they are old. When clade age is accounted for using phylogenetic methods, diversification is not correlated with warmth or *lebensraum*. Instead, the number of different environments, and presumably, different selective pressures, to which the clade is exposed seem to play a key role. For all the wonderful luxuriance of the tropics, it may actually be the diverse pressures of changing seasons that provide selective pressure for speciation. Perhaps, like 19th century explorers returning home, it is time to appreciate the special qualities of temperate environments. The key to understanding major ecological patterns may be on our own doorsteps.

Chapter 2: Functional diversification under contrasting seasonality in three Australian rainforest communities

Introduction

Diversity is often seen very differently by ecologists and systematists: one of the goals of ecology is to measure functional diversity; for example, though guilds, trophic levels, and vegetation types. Systematists tend to measure diversity phylogenetically, through disparity in shared evolutionary history. While these two definitions of diversity are often conflated, they are not the same (Tilman *et al.* 1997; Loreau *et al.* 2001; Hooper *et al.* 2005; Petchey & Gaston 2006).

The tropics are famously far more speciose than temperate regions (Hawkins 2001; Willig, Kaufman, & Stevens 2003; Mittelbach *et al.* 2007). Yet whether the dramatic species richness of the tropics is reflected in equally high functional diversity remains an open question (Cabido 2001; Hooper *et al.* 2005; Condit *et al.* 2006). Under a model in which all niches have the same breadth, and are evenly spaced along trait axes, higher functional diversity is a necessary correlate of species richness. Yet, there is evidence that this may not be the case. Data from a highly diverse tropical forest (Kraft, Valencia, & Ackerly 2008) suggest trait diversity lower than that found in communities with less than one-tenth the diversity (Cornwell & Ackerly 2009). This suggests that functional diversity within communities may be due to interactions with the environment, rather than interactions between species.

Within any community, the spectrum of functional traits observed results from the interplay of two different forces. The first is local adaptation, with functional diversity arising through adaptive evolution. The second is phylogenetic niche conservatism (Harvey & Pagel 1991; Ricklefs & Latham 1992; Peterson, Soberón, & Sánchez-Cordero 1999; Ackerly *et al.* 2002; Webb *et al.* 2002; Ackerly 2004; Wiens 2004). This is the tendency for taxa to track environments to which they are already suited. In a community of well-adapted competitors, dispersules are most likely to prosper in microsites resembling those of their parents—i.e., sites to which they are already adapted. As a result, phylogenetic niche conservatism results in the maintenance of similar trait values across communities (Travis 1989; Harvey & Pagel 1991; Ackerly 2003, 2004). Although it is generally acknowledged that both forces operate, the relative degree to which each prevails is often difficult to quantify. At larger scales, dispersules may not always reach optimal sites (Ackerly 2003). The availability of empty, but suboptimal, sites leaves room for adaptive change in other lineages. By contrast, phylogenetic niche conservatism has been observed even at continental scales (Lord, Westoby, & Leishman 1995; Prinzing 2001; Forget *et al.* 2007).

This study explores these interrelated facets of biological diversity from two different angles. First, I examine how functional diversity is related to limiting environmental conditions and species richness; second, I investigate the relative contribution of phylogenetic niche conservatism and local adaptation to differences in functional diversity under three different seasonality regimes.

In Australia, thin-leaved, closed-canopy forests are found in three different climates: as wet tropical forest, seasonally dry tropical forest, and wet temperate forest. These notophyll forests grow along the east coast of Australia, and form "environmental islands" within the surrounding Eucalyptus- and Acacia- dominated sclerophyll woodlands (Webb 1959, 1968; Webb & Tracey 1981). They are an excellent system for studying evolutionary processes because there has been minimal exchange with other floristic regions, and because each community has adapted to different seasonal limitations (Werren & Kershaw 1991; Hill 2004; Crisp *et al.* 2009). These conditions are not found elsewhere, particularly in the northern hemisphere where tropical to temperate gradients represent an exchange between Gondwanan floras, Holarctic or Laurasian floras, and possibly other groups as well (Takhtajan, Crovello, & Cronquist 1986). The different climatic regimes in each community form contrasts between temperature, seasonality, and water availability (Table 7).

Table 7: Contrasts in seasonality conditions between the three sites

Wet tropical	*Dry tropical*	*Wet temperate*
←Tropical→		Temperate
Aseasonal	←Seasonal→	
←Wet	Dry	Wet→

The importance of functional trait variance and species co-existence

Within communities, variance in physiological function is an important indicator of ecological processes. Low variance indicates that a narrow range of functional values are viable; this has been equated with habitat filtering (van der Valk 1981; Keddy 1992; Díaz, Cabido, & Casanoves 1998; Weiher & Keddy 2001; Cornwell, Schwilk, & Ackerly 2006; Cornwell & Ackerly 2009). High trait variance can indicate multiple different strategies coexisting at the same site. This indicates resource partitioning (Chesson 2000; Poorter 2007), as either even trait spacing (Ackerly & Cornwell 2007; Cornwell & Ackerly 2009) or limiting similarity (MacArthur & Levins 1967; Pacala & Tilman 1994).

Global data sets show changes in variance across biomes that appear to be independent of trends in means (Figure 4). For example, leaf lifespan shows little change in mean across environments, but large changes in variance. In this case, high variance reflects the coexistence of multiple strategies: the presence of both long lived evergreen and short lived deciduous leaves in response to seasonality at higher latitudes (Wright *et al.* 2005). Differences in variance are also observed in other traits: in plant height, high variance at high latitudes is largely explained by the coexistence of herbs and trees (Moles *et al.* 2009), although the selective pressures for this difference are not clear. The greater variance in wood density at midlatitudes is likely due to the coexistence of dense-wooded evergreens and water-storing drought-deciduous trees in dry tropical forests (e.g., Choat *et al.* 2005). These patterns suggest that the coexistence of multiple successful strategies may correspond to environmental limitations of seasonal cold and drought respectively, leading to Hypothesis 1: Functional trait variance will be higher in seasonal environments, indicating a spectrum of coexisting functional strategies. As a corollary, this variance will correspond to the most limiting conditions in each of the two seasonal environments—drought and cold, respectively. Specifically, leaf traits are expected to show greater variance at the two seasonal sites. In particular, leaf size and leaf dry mass per unit area are expected to show a greater range of values at both seasonal sites, in response to temperature and drought (Givnish 1987; Ackerly & Reich 1999; Moles *et al.* 2011). By contrast, wood density is expected to show a peak in variance at the dry site only, corresponding to the

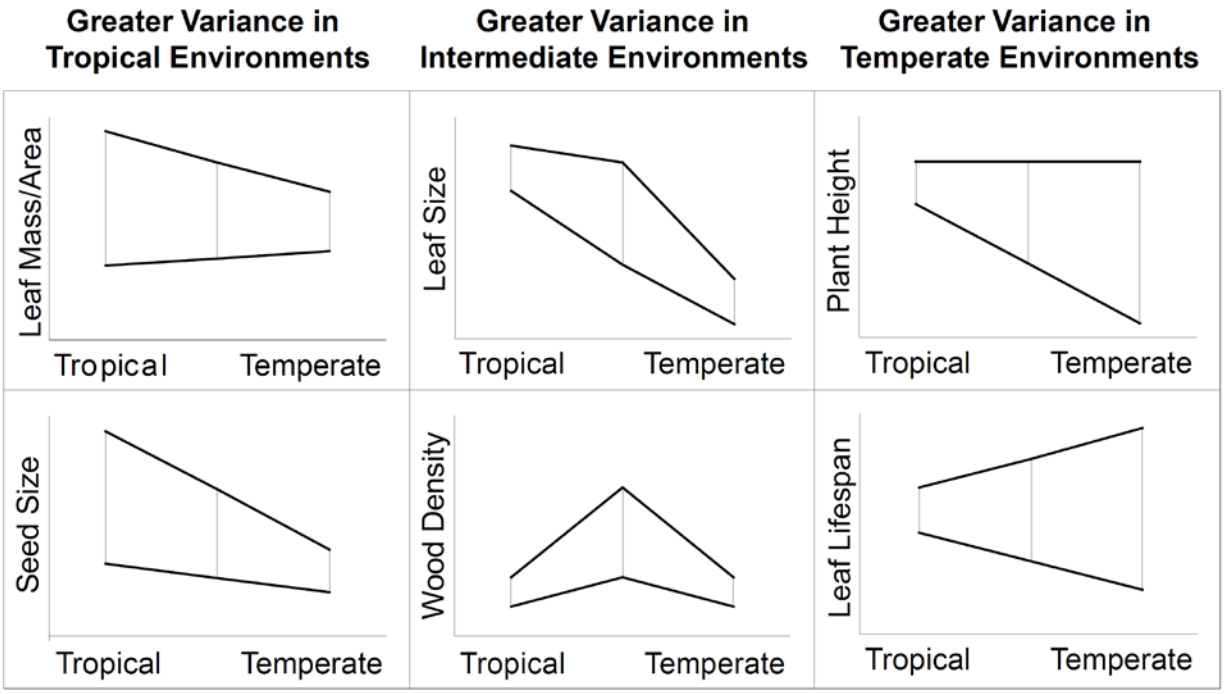


Figure 4: Global trends in functional trait mean and variance Data sources: LMA and leaf lifespan (Wright *et al.* 2005), leaf size (Moles *et al.* 2011), height (Moles *et al.* 2009), seed size (Moles *et al.* 2007), wood density (Chave *et al.* 2009).

coexistence of drought-deciduous and drought-green trees, strategies associated with spongy and dense wood respectively.

The relative contribution of local adaptation and phylogenetic niche conservatism to functional trait differences

The processes by which variation arises are also important in understanding differences in trait values across sites. Phylogenetic niche conservatism, the process by which taxa track environments to which they are already well adapted, is supported by a wealth of evidence (Harvey & Pagel 1991; Ricklefs & Latham 1992; Peterson, Soberón, & Sánchez-Cordero 1999; Ackerly *et al.* 2002; Webb *et al.* 2002; Ackerly 2004; Wiens 2004). At the same time, it is clear that in some situations, adaptation to local conditions also plays a role (Ackerly 2003). While niche conservatism appears to dominate at local scales, at larger scales, within and across continents, it is not clear how much variation is due to each process (Ackerly 2003, 2004; Crisp *et al.* 2009). This project investigates the relative contribution of niche conservatism and local adaptation to changes in trait values in widespread clades that occur at all three sites. This leads to Hypothesis 2: Traits relating to relative growth rate and life history strategy will be more conserved than traits relating to water use and cold tolerance. At the large spatial and deep temporal scales examined here, local adaptation is expected to dominate. Nonetheless, a proportion of trait variation among widespread clades is expected to be explained by phylogenetic relationships. Greater conservatism is predicted in wood characters, SLA, and leaf dry matter content, which relate to growth strategies and successional status, than in leaf size and sapwood to leaf area ratio, which relate to cold and drought tolerance.

Methods

Site selection

Three Australian evergreen notophyll forest sites were chosen to exemplify the contrasts in climate investigated by this study: wet tropical, seasonally dry tropical, and wet temperate (seasonally cold) forest communities. Within Australia, notophyll forest does not occur at cold, seasonally dry sites. Each site is located in a protected area, to minimize industrial and agricultural impacts and maximize usefulness of this work in the context of future surveys. The sites were also chosen for their past research history. The wet tropical site is located at the Australian Canopy Crane Research Facility, a long-term ecological research site established by a consortium of Australian universities in 1998. Seasonally-dry tropical forest occurs west and south of the wet tropics. The second site, at the Forty Mile Scrub National Park, has been used in a number of projects and surveys (Ash 1988; Conn & Broen 1993; Fensham 1995, 1996a) and there is a tagged plot established by the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO; Unwin & Kriedemann 1990) and utilized by the Tropical Biomes in Transition project (TROBIT, e.g., Feldpausch *et al.* 2010) of the University of Leeds. The Forty Mile Scrub is notable for a large area of contiguous dry forest. Seasonally-dry tropical forest is one of the most endangered habitats in Australia, making it difficult to locate large patches and nearly impossible to locate pristine forest (Fensham 1996b; Fensham & Skull 1999). The Forty Mile Scrub site is heavily invaded by *Lantana camara*, which has been excluded from this analysis (Fensham, Fairfax, & Cannell 1994). In Tasmania, samples were collected from Mount Field National Park. Mount Field also has a long history of ecological research (ex., Smith 1981; Gibson & Kirkpatrick 1985; Minchin 1989; Brodribb & Feild 2000; Jordan, Brodribb, & Loney 2005; Blackman, Brodribb, & Jordan 2011). It contains patches of temperate wet

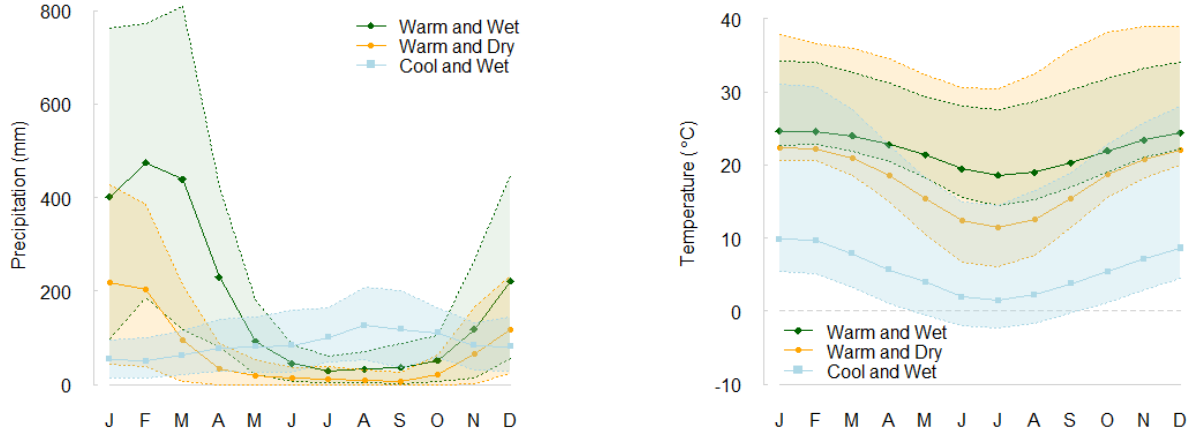


Figure 5: Study site climates Solid lines with symbols show monthly means (precipitation) and monthly mean minimum (temperature) for the period 1970–2010. At left, shaded area and dotted lines show the 10th and 90th percentiles for monthly rainfall over the same period. At right, the shaded area shows the difference in daily temperature range, represented as the difference between the 10th percentile of minimum temperature, and the 90th percentile of maximum temperature, for each month over 40 years. Each figure is the average of the three closet weather stations in comparable climate zones to the study site. Although a standard data range of 1970–2010 was used for all sites, not all sites have continuous data during this period. In some cases, two nearby sites were combined (e.g., a post office site that was closed in the 1990s was combined with an airport site for the same township opened in the 2000s.)

forest of varying size. Transects were taken at several different locations within the park to account for the patchy nature of the vegetation.

Each of the sites surveyed had at least one climatic parameter in common with another site, but each also had a unique combination of climatic parameters (Table 7). Although there is temperature and rainfall seasonality at the wet tropical site, the temperature never drops below freezing and a minimum monthly rainfall of >80 mm ensures adequate soil moisture throughout the year (Figure 5). Thus, this site does not experience an unfavorable season. The seasonally dry site also does not experience freezing, but there is a three to five month dry season. At the cold site, rainfall is comparable to the wet site, with annual means of ~2300 mm and ~3400 mm, respectively. Tropical environments typically need a higher rainfall than temperate sites to have comparable water availability, due to greater evaporation under warmer conditions (Whittaker 1975). The temperate site experiences >100 potential frost days per year (Australian Government Bureau of Meteorology 2011). Thus differences in rainfall and temperature seasonality form the major contrast in abiotic conditions between these sites.

Site surveys and sampling design

Sampling took place in two stages. Taxonomic diversity varied greatly between sites, and exhaustive sampling at any site was beyond the scope of this project. As an alternative, a representative random sample of the community was collected at each location. This was done by surveying ten randomly-placed 50 m × 2 m Gentry transects (0.1 ha, Gentry 1982, 1988). These were distributed between two separate patches of the habitat type of interest, at the Mt. Field and Forty Mile sites, and between lowland and hillside sites at the Canopy Crane. From these surveys, a community species list of all vascular plants found in the transects was constructed. Functional trait samples were then collected for each species on this list.

Sample size and replication are important considerations in any study of functional traits. The goal of accurately estimating trait values must be balanced against the resources available (Hurlbert 1984). In this study, the statistical design is replicated at the species level, following the allocation of effort suggested by Cornelissen et al. (2003). This sacrifices precision in quantifying the mean for each individual, but focuses sampling effort on the comparison of greatest interest. For each species of interest, five individuals were sought from the area in and around the transects, and a single sample was taken from each. Sample size varied from five (or in a few cases where it was necessary to use separate individuals for leaf traits and wood traits, six or seven), to zero for a few species that occurred as juveniles or seedlings in the transect, and for which no adult individual was ever found. Of the 261 taxa sampled for functional traits across the three sites, 52% were represented by at least five individuals; 76% were represented by at least three individuals.

Trait collection

For woody plants, a branch ~0.5–2 cm in diameter was collected from the highest light conditions in which the species was found. Canopy branches were collected using the canopy crane at the warm and wet site, and pole pruners at the other two sites. The branch was tagged with locality information, and placed in a large plastic bag with moist paper toweling; the bag was also

periodically misted through the day. A number of bags were generally collected in one day, then transported to the laboratory facility. Stem ends were cut under water, and then each branch was placed with the cut end immersed in water. The container and the branches were covered with a plastic bag. The branches were stored for at least 24 hours in order to ensure full hydration (Cornelissen *et al.* 2003). Each branch was then removed from water and samples were taken from the proximal end in the following order: the submerged portion of the branch was removed and discarded. A section was then taken for wood density analysis, followed by a sample for sapwood area. Both samples were stored at 4°C in polyethylene plastic bags with a moist paper towel for later processing. At least two leaves were selected and removed. For Acacia, Phyllocladus, and Callitris a leaf analog was used instead of a leaf, as suggested by Cornelissen (2003). One leaf was measured from petiole to tip, and leaf length was recorded; then the sample was weighed. For the other sample, a minimum photosynthetic unit, consisting of either one or more leaves without the petiole, or one or more individual leaflets, was selected. The petiole or rachis was removed with a razor blade and discarded before weighing. Each leaf was pressed under glass and photographed on a light table with a scale. Thick petioles or rachides were photographed separately without the glass in place. In some cases, leaves were cut into smaller portions to flatten them or to fit them in the photographic field. Leaves were then placed in labeled envelopes, and dried in a drying oven for either 72 h at 60°C or 48 h at 80°C, to accommodate other users sharing the ovens. The rest of the branch was stripped of all remaining leaves, which were placed in a paper bag and dried at the same temperature.

The wood samples were processed as follows: the wood density sample was trimmed to 2.5 cm and split in half using a razor blade and a hammer. One half was saved, and the pith was removed using woodcut tools. The other half was discarded. The volume of the remaining half, with no pith, was fixed to a thin needle inserted into a wooden dowel, and suspended over the balance using a retort stand. The sample was then submerged in a beaker of water placed on the balance. The balance used had an animal measurement function, which averages sample weight over a predetermined period (in this case, 30 seconds.) This averaging function was used to determine the amount of water displaced. The sample was then removed, placed in an envelope, and dried at 60–80° for transport back to Canberra. The anatomy sample was trimmed, and cross-sectional diameter (excluding bark) and pith diameter were measured using digital calipers (Mitutoyo America Corporation).

At the end of the field season, dried leaf and wood samples were analyzed at host labs in Canberra, ACT, and Richmond, NSW, Australia. Leaf samples—both single leaves and all other leaves distal to the sample cut—were returned to the drying oven at 60°C for at least three days before dry weight was measured. Wood samples were dried at 110°C following Williamson & Wiemann (2010) before weighing. The leaf area photographs were processed using the Image J (U.S. National Institutes of Health, Bethesda, Maryland, USA) particle analysis module. Functional trait values were calculated as indicated in Table 8.

Table 8: Traits used in the study

	Definition	*Calculation*	*Units*	*Log-normal*
Leaf Length (p)	Leaf length, with petiole	Length of one leaf	cm	yes
Leaf Area (p)	Leaf area, with petiole	Surface area per leaf	mm^2	yes
Leaf Area (np)	Leaf area, without petiole	Surface area per leaf	mm^2	yes
SLA (p)	Specific leaf area, with petiole	Leaf area/leaf dry mass	$m^2\,kg^{-1}$	yes
SLA (np)	Specific leaf area, without petiole	Leaf area/ leaf dry mass	$m^2\,kg^{-1}$	yes
LDMC (p)	Leaf dry matter content, with petiole	Leaf dry mass/leaf fresh mass	$mg\,g^{-1}$	no
LDMC (np)	Leaf dry matter content, without petiole	Leaf dry mass/leaf fresh mass	$mg\,g^{-1}$	no
WD	Wood density	Stem dry mass/stem volume	$mg\,mm^{-3}$	no
HV	Huber value (leaf area:sapwood area)	Leaf area/sapwood area	-	yes
Pith-SW Ratio	Ratio of pith area to sapwood area	Pith area/sapwood area	-	no
Percent Pith	Pith as a percent of stem cross-section	Pith area/stem cross-sectional area	-	no

Selection of wide-spread clades

A community phylogeny of all taxa from all sites was created using Phylomatic (Webb & Donoghue 2005; Webb, Ackerly, & Kembel 2008). Sites were mapped on to the topology, and the smallest clades including at least one taxon from all three sites were selected. This resulted in 18 clades of varying size (Appendix A). In most cases, the comparison is not balanced between sites; the most unbalanced comparison is the Magnoliales-Laurales clade with 18 wet tropical taxa, and only a single taxon at the other two sites. In many cases, the greater taxonomic diversity of the two tropical sites makes selecting clades which are both balanced and inclusive (i.e., not paraphyletic with respect to the study taxa) not possible. Nonetheless, the average difference in number of taxa between each clade by site combination is only 2.4. The depth of these clades also differs considerably; the estimated minimum age of the most recent common ancestor varies from ~30 Ma for Clade 8, the ziziphoid Rhamnaceae, to greater than 130 Ma for Clade 17, Magnoliales-Laurales (age estimates based on interpolation from Wikström, Savolainen, & Chase 2001; Webb & Donoghue 2005; Webb, Ackerly, & Kembel 2008).

Statistical analysis

Analysis was carried out in the R statistical environment (R Development Core Team 2011). A single mean was calculated for each species at each site. A number of the traits, particularly those relating to area, were strongly log normal and were log-transformed before further analysis (Table 8). Differences in variance between sites were assessed using Levene's test for equal variances, and differences in means between sites were tested by ANOVA, using a White corrected-covariance matrix to account for heteroscedasticity between groups as demonstrated by Levene's test. For consistency, this correction was used even where Levene's test indicated no significant differences, as the majority of traits had some difference in variance between sites. Both tests were implemented in the car package for R (Fox & Weisberg 2001). Tukey's HSD was used to establish differences in mean trait value between particular sites. To test which sites differed significantly in variance, multiple pairwise instances of Levene's test was also used, restricted to the comparison of interest. Pairwise Kolmogorov-Smirnov tests were also used to compare differences in distribution between the two sites. This test is sensitive to differences mean, variance, and the shape of distributions that are not always detected by the other two tests. For both multiple Levene's and multiple Kolmogorov-Smironov tests, resulting p values were adjusted for multiple comparisons using the

method of Holm (1979). Except where noted, the values shown include traits for all vascular plants included in the study.

Relative phylogenetic niche conservatism between the three sites was assessed using a randomized null model approach. The observed data for each trait were compared to two different models. The first is a model of maximum phylogenetic niche conservatism in which clades are assigned to trait values in order at each site, such that the relative rank of each clade is the same at each site, with no crossover in clades between sites. This represents the maximum degree of conservatism possible, given the observed data. ANOVA on this model quantifies the maximum amount of variance in trait values that could be attributed to clade structure, based on the percent of total sums of squares. The second model is a random null model, in which clades and trait values are randomly shuffled (sampled without replacement) within each site. ANOVA was performed on a population of 100 such random communities, and sums of squares were averaged across the entire sample. This represents the amount of variation attributed to clades that is expected to arise by chance. These two measures were then compared to the observed amount of variation due to clades, measured as the Clade Sum of Squares in an ANOVA on the observed data. Observed conservatism as a percent of total possible conservatism, called the Conserved Index (CI), was calculated as

$$\frac{SS\ Clade_{observed} - SS\ Clade_{random}}{SS\ Clade_{conserved} - SS\ Clade_{random}} \qquad \textit{Equation 1}$$

where SS $Clade_{observed}$, SS $Clade_{conserved}$, and SS $Clade_{random}$ are the Clade Sum of Squares for the observed data, the maximally-ordered data, and the mean of 100 randomly shuffled populations, respectively. Significance for the CI was calculated by comparing the SS $Clade_{observed}$ to the results of 1000 random shuffles. The proportion of results produced with values larger than the observed were treated as a one-tailed test.

Local adaptation is reflected in two other aspects of these analyses. Because taxa are shuffled within sites for both the conserved and random models, the main effect of site is held constant across all comparisons. It represents the shared aspect of local adaptation across clades—the overall average shift in trait values between communities. This can be conceptualized as the β trait value—the difference between communities (Cornwell & Ackerly 2009). The site by clade interaction reflects the extent to which individual clades have adapted differently to the gradient, as well as shifts in their position within the community. It is perhaps easiest to conceptualize these two aspects graphically: shifts in position within the community would be represented by crossing lines on an interaction graph such as Figures 8 and 9. This pattern indicates clades do not conserve their positions in the two communities; this is equivalent to a change in α trait value (Cornwell & Ackerly 2009). Adaptation of two individual clades to local conditions would be visualized as two non-crossing, but not parallel lines in an interaction graph, also known as an ordinal interaction. This indicates that local adaptive shifts are stronger in some clades.

In order to test the robustness of this new measure, two methods of calculating CI were compared. In the first (Model A), ANOVA is calculated on taxon means for each site, i.e., one mean for each

species listed in Appendix A. This leads to unbalanced sample sizes between sites for some clades, but allows the calculation of a site by clade interaction. The second (Model B) calculates ANOVA on a single mean for each clade at each site. Without variance within clades, it is not possible to calculate an interaction; this model includes only the main effects of clade and site.

Results

Site surveys

At each site, surveys captured ~10% of the total local flora, as estimated by species lists available for each of the three national parks (Queensland Department of Environment and Resource Management 2009; Parks & Wildlife Tasmania 2010). Although the Canopy Crane surveys appear to capture very slightly more, the highly diverse local flora is also the most likely to contained undescribed taxa. Markedly different levels of taxonomic diversity were found at each of the three sites (Table 9). All vascular plants were included in the surveys and subsequent analyses. A number of different fern taxa were represented at each site, with the highest diversity of ferns occurring at the wet and seasonally cold site, Mt. Field. Gymnosperms were also represented at each of the three sites, by conifers at the two seasonal sites, and by two cycads at the wet tropical site. There was a distinct latitudinal gradient in the presence of compound-leaved taxa, with many more found at the tropical sites, particularly the tropical wet site. Although one deciduous species, *Nothofagus gunnii*, is known from Mt. Field, it did not occur in any of the survey transects and was not visible from the transect lines.

Functional trait variation under contrasting seasonality conditions

The first aim of the study was to examine how whether communities with different seasonality regimes had different ranges of coexisting functional traits. Both changes in mean, and changes in variance, were considered. Although analyses compared variance between sites, figures show standard deviation (the square root of variance), which has the same units as trait means, for ease of comparison. Shifts in mean and standard deviation across all three sites are shown in Figures 6 and 7, and corresponding ANOVA results in Table 10. Results are shown for both the complete community, including all vascular plants, and for angiosperms only. There were significant differences in mean values for all traits except SLA. Variance was significantly different between sites for all leaf traits. Variance was not significantly different between sites for any of the wood traits except pith as a percent of stem cross-sectional area. Pairwise differences between trait distributions, means, and variances (Table 11) are discussed below.

Table 9: Summary of surveys at the three sites The sites are wet tropical (Canopy Crane), seasonally dry tropical (Forty Mile Scrub) and wet temperate (Mt. Field.) The local species pool for each community was estimated from the species lists available for Mt. Field National Park for the seasonally cold sites, and Daintree National Park for the tropical wet site. Forty Mile Scrub National Park has a comparatively small total area; the species pool for this site was compiled from lists for both the Forty Mile Scrub and the nearby Undara Volcanic National Park.

						Compound-			*Families in common:*		
	Species surveyed	*Local species*	*Ferns*	*Gymnosperms*	*Angiosperms*	*leaved taxa*	*Deciduous taxa*	*Families*	*CC*	*FM*	*MF*
Canopy Crane	149	1285	8	2	139	34	0	64	-	24	16
40 Mile Scrub	65	638	4	1	60	10	3	40	24	-	12
Mt. Field	47	434	13	1	33	6	0	32	16	12	-

Leaf functional traits consistently showed greater variance at the temperate site than at the wet tropical site (Figure 6; Table 11). Leaf area, SLA, and leaf dry matter content were measured both for entire leaves (with petiole, p) and minimum photosynthetic units (leaflets or leaves without petiole, np). Very similar patterns of significance and magnitude were observed for both treatments. The wet tropical site was characterized by large leaves with high water content, with low variance in both traits. The temperate site had, on average, smaller leaves with a lower water content, and greater variance in both traits. Like the temperate site, the dry tropical site had small leaves. However, it had intermediate variance in leaf size: similar to the temperate site, for minimum photosynthetic units, but not significantly different from either site for whole leaves including petioles. A test on leaf size variance restricted to only angiosperms found significant difference between the wet tropical and other two sites ($p = 0.008$ and $p = 0.022$ for CC-FM and CC-MF, respectively) but no difference between the two seasonal sites ($p > 0.5$). This suggests that the pattern for all vascular plants may be strongly influenced by the presence of large-leaved ferns at the temperate site, such as Dicksonia antarctica, which, with an average leaf length over 3 m, was an outlier even in log-transformed data. Mean SLA at all three sites was statistically indistinguishable. Variance was significantly higher at the two seasonal sites than at the aseasonal site.

Table 10: Differences in mean and variance of functional traits between sites Left, *p* values shown are for ANOVA with heteroscedasticity-corrected covariance matrices (see Methods.) Right, Levene's test of equal variances. Sample size *(n)* shows number of species means.

			All Taxa							
	n	*Units*	*ANOVA: differences in mean*				*Levene's test: differences in variance*			
			F	*df*	*p*		*F*	*df*	*p*	
Leaf Length	231	log cm	28.02	2, 228	$2.83e^{-16}$	***	9.77	2, 228	$8.47e^{-5}$	***
Leaf Area, with petiole	234	log m^2	58.90	2, 231	$< 2.2e^{-16}$	***	3.50	2, 231	0.032	*
Leaf Area, no petiole	238	log m^2	73.75	2, 235	$< 2.2e^{-16}$	***	6.81	2, 235	0.001	**
SLA, with petiole	233	log $m^2 kg^{-1}$	0.87	2, 230	0.418		5.47	2, 230	0.005	**
SLA, no petiole	238	log $m^2 kg^{-1}$	1.17	2, 235	0.312		4.77	2, 235	0.009	**
Leaf Dry Matter, with petiole	253	mg g^{-1}	7.36	2, 250	$7.82e^{-4}$	***	5.33	2, 250	0.005	**
Leaf Dry Matter, no petiole	261	mg g^{-1}	7.42	2, 258	$7.34e^{-4}$	***	3.85	2, 258	0.022	*
Wood Density	190	mg mm^{-3}	10.25	2, 187	$6.00e^{-5}$	***	2.03	2, 187	0.134	
Huber Value	178	-	35.18	2, 175	$1.44e^{-13}$	***	0.14	2, 175	0.866	
Pith-to-Sapwood Ratio	196	-	25.46	2, 193	$1.54e^{-10}$	***	1.30	2, 193	0.275	
Pith as a Percent of Stem	196	-	25.64	2, 193	$1.33e^{-10}$	***	4.15	2, 193	0.017	*
			Angiosperms							
	n	*Units*	*ANOVA: differences in mean*				*Levene's test: differences in variance*			
			F	*df*	*p*		*F*	*df*	*p*	
Leaf Length	205	log cm	45.30	2, 202	$< 2.2e^{-16}$	***	4.73	2, 202	0.00982	**
Leaf Area, with petiole	208	log m^2	66.42	2, 205	$< 2.2e^{-16}$	***	1.79	2, 205	0.1689	
Leaf Area, no petiole	211	log m^2	73.93	2, 208	$< 2.2e^{-16}$	***	6.70	2, 208	0.00151	**
SLA, with petiole	207	log $m^2 kg^{-1}$	1.02	2, 204	0.3636		5.28	2, 204	0.00582	**
SLA, no petiole	211	log $m^2 kg^{-1}$	2.81	2, 208	0.06263		4.29	2, 208	0.01488	*
Leaf Dry Matter, with petiole	225	mg g^{-1}	6.22	2, 222	0.00234	**	4.14	2, 222	0.01719	*
Leaf Dry Matter, no petiole	232	mg g^{-1}	7.26	2, 229	$8.75e^{-4}$	***	2.72	2, 229	0.06789	
Wood Density	188	mg mm^{-3}	9.79	2, 185	$9.07e^{-5}$	***	2.24	2, 185	0.1093	
Huber Value	176	-	34.06	2, 173	$3.38e^{-13}$	***	0.11	2, 173	0.9002	
Pith-to-Sapwood Ratio	194	-	24.66	2, 191	$2.97e^{-10}$	***	1.39	2, 191	0.2507	
Pith as a Percent of Stem	194	-	24.87	2, 191	$2.56e^{-10}$	***	4.12	2, 191	0.01773	*

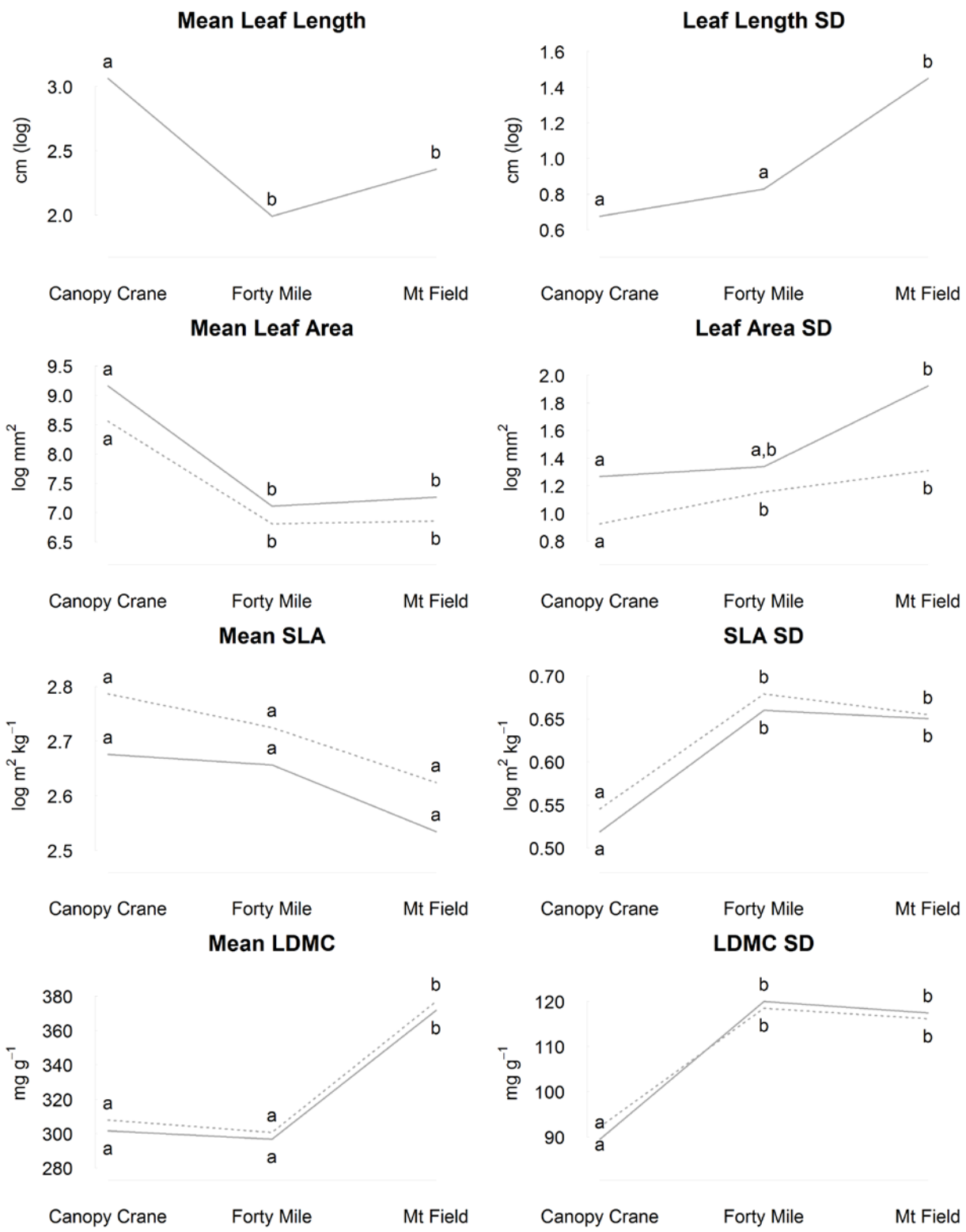


Figure 6: Changes in mean and standard deviation of leaf traits Changes in mean are shown at left; changes in standard deviation (square root of variance) are shown at right. Standard deviation was chosen for display because it is expressed in the same units as mean and can be more easily compared. Data based on entire leaves including petioles are shown as solid lines; data for minimum photosynthetic unit without petiole are shown as a dashed line. Lowercase letters indicate significant differences between groups within each trait type; for significance levels, see Table 11.

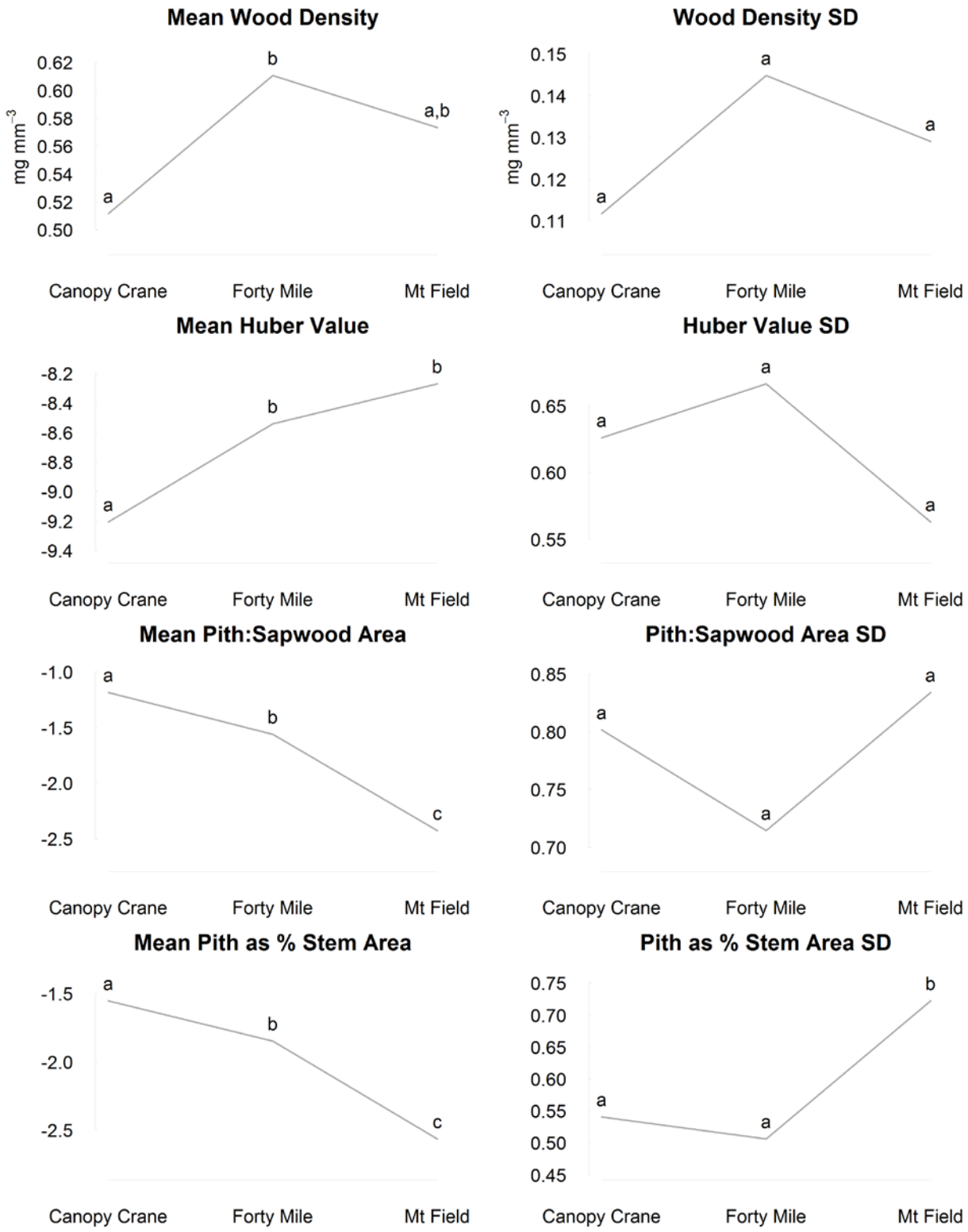


Figure 7: Changes in mean and variance of wood traits Changes in mean are shown at left; changes in standard deviation (square root of variance) are shown at right. Standard deviation was chosen for display because it is expressed in the same units as mean and can be more easily compared. Lowercase letters indicate significant differences between groups within each trait type; for significance levels, see Table 11.

Table 11: Pairwise differences between sites Differences in distributions (Kolmogorov-Smirnov), means (Tukey), and variance (Levene) between three sites. Kolmogorov-Smirnov and Levene adjusted for multiple comparisons.

	Distributions			Means			Variance		
	CC-FM	CC-MF	FM-MF	CC-FM	CC-MF	FM-MF	CC-FM	CC-MF	FM-MF
Leaf Length	$3.3e^{-16}$ ***	$7.0e^{-6}$ ***	0.368	$1.0e^{-8}$ ***	$4.5e^{-4}$ ***	0.157	0.074	$9.6e^{-5}$ ***	0.026 *
Leaf Area (p)	$1.0e^{-15}$ ***	$6.9e^{-15}$ ***	0.805	$3.3e^{-14}$ ***	$1.5e^{-12}$ ***	0.845	0.389	0.038 *	0.232
Leaf Area (np)	$6.7e^{-16}$ ***	$2.0e^{-13}$ ***	0.710	$2.9e^{-14}$ ***	$3.3e^{-14}$ ***	0.970	0.030 *	0.002 **	0.311
SLA (p)	0.607	0.132	0.596	-	-	-	0.017 *	0.019 *	0.976
SLA (np)	0.261	0.059	0.895	-	-	-	0.024 *	0.042 *	0.899
LDMC (p)	1.000	0.003 **	0.010 **	0.947	$2.2e^{-4}$ ***	$5.6e^{-4}$ ***	0.012 *	0.026 *	0.900
LDMC (np)	0.567	0.008 **	0.008 **	0.886	$2.7e^{-4}$ ***	$4.6e^{-4}$ ***	0.013 *	0.043 *	0.853
Wood Density	$6.4e^{-6}$ ***	$3.7e^{-3}$ **	0.162	$1.3e^{-5}$ ***	0.058	0.428	-	-	-
Huber Value	$5.3e^{-8}$ ***	$2.8e^{-9}$ ***	0.389	$1.7e^{-8}$ ***	$3.3e^{-10}$ ***	0.175	-	-	-
Pith Ratio	0.005 **	$7.0e^{-8}$ ***	$2.4e^{-5}$ ***	0.013 *	$6.0e^{-12}$ ***	$1.6e^{-5}$ ***	-	-	-
Pith Percent	0.002 **	$4.0e^{-8}$ ***	$9.5e^{-5}$ ***	0.006 **	$1.0e^{-8}$ ***	$4.6e^{-7}$ ***	0.401	0.031 *	0.025 *

Contrasting patterns were observed in wood traits, which generally did not show significant differences in variance between the three sites (Figure 7; Table 11). Mean wood density was lowest at the aseasonal site, and highest at the dry site; the temperate site was intermediate. There was no statistical difference in variance between the sites. Huber value, the ratio of sapwood to downstream leaf area, was significantly lower at the tropical site, with no statistical difference in variance. Pith traits followed a slightly different pattern: means from all three sites were statistically different, with the dry tropical site intermediate. There was no significant difference in pith:sapwood ratio variance between any of the sites. Pith as a percent of stem area had greater variance at the temperate site than at the two tropical sites.

Pairwise comparisons of trait distributions, means, and variance between the sites tend to reflect either the contrast between aseasonal and seasonal environments, or tropical and temperate environments (Table 7). For all traits in which variance differed between sites, it was higher at the temperate site than at the wet tropical site. The dry tropical site usually also showed greater variance relative to the wet tropical site, with two exceptions. Variance in leaf length and percent pith area both showed a tropical-to-temperate split, with greater variance at the temperate site.

Phylogenetic niche conservatism and local adaptation in functional traits

The second goal of this study was to examine the relative contributions of niche conservatism and local adaptation to trait values observed at each site. Eighteen widespread clades occurring at all three sites were included; see Methods, this chapter, and Appendix A for details. The relative ability of clade membership, community membership, and their interaction (for Method A) to explain variation observed was explored through ANOVA and is shown in Table 12. In the context of this analysis, significant clade effects describe trait conservatism; both significant site effects and significant interactions describe local adaptation. Significant site effects describe differences in mean between the sites; significant interaction effects describe changes in the differences between clade means which are dependent on site. Interactions reflect changes in relative magnitude of clade effects, including greater adaptation in some clades, or crossover in the relative rank of clades at

different sites. However, these should be interpreted with caution; in all cases, ANOVA using pure-conservatism null models also showed a significant interaction ($p > 0.005$).

In the presence of a significant interaction effect, the relative change in rank can be determined by examining crossover in clades between sites. Figures 8 and 9 show crossover in trait values between sites for leaf and wood traits respectively. Conservatism is indicated by parallel lines connecting trait means; the greater the slope of each line, the more change has taken place between environments. Both figures demonstrate that crossover—particularly a small amount of crossover, within either the upper, or lower, half of the trait distribution—is common; none of the traits examined here show pure conservatism. Instead, they show a mix of relative conservatism and dramatic local adaptation.

In order to quantify the amount of variation among widespread clades at different sites, the observed results were compared to two different null models. The first represented perfect conservatism—no crossover in the relative rank of trait values by clade between communities. The second represented a distribution of completely randomized communities, with only a minimal amount of variation explained by clade. The amount of variation that can be attributed to phylogenetic niche conservatism is expressed as Conserved Index (CI, Equation 1). CI for each trait is shown with significance in Table 13.

Table 12: Relative effects of clade, site, and site-by-clade interaction Model A: Means of each taxon within each clade at each site; Model B: a single mean for each clade at each site. Calculations for each trait are restricted to clades sampled at all three sites. For clade and taxon sample sizes, see Table 13.

Model A

	Site				*Clade*				*Site × Clade*				*Residuals*	
	Sum Sq	*df*	*F*	*p*	*Sum Sq*	*df*	*F*	*p*	*Sum Sq*	*df*	*F*	*p*	*Sum Sq*	*df*
Leaf Length	19.53	2	36.69	$3.2e^{-9}$ ***	8.14	8	3.82	0.003 **	9.18	16	2.15	0.030 *	9.05	34
Leaf Area (p)	94.56	2	64.33	$< 2.2e^{-16}$ ***	29.21	14	2.84	0.002 **	49.52	28	2.41	$1.2e^{-3}$ **	58.80	80
Leaf Area (np)	79.82	2	53.36	$2.17e^{-15}$ ***	23.16	14	2.21	0.014 *	52.01	28	2.48	$8.6e^{-4}$ ***	59.08	79
SLA (p)	0.88	2	1.84	0.165	10.75	14	3.21	$4.8e^{-4}$ ***	13.51	28	2.02	0.008 **	19.13	80
SLA (np)	1.86	2	4.06	0.021 *	12.66	14	3.95	$4.2e^{-5}$ ***	14.04	28	2.19	0.004 **	18.09	79
LDMC (p)	$1.7e^{5}$	2	13.44	$7.5e^{-6}$ ***	$7.4e^{5}$	17	6.86	$2.6e^{-10}$ ***	$3.9e^{5}$	34	1.80	0.015 *	$5.8e^{5}$	92
LDMC (np)	$2.0e^{5}$	2	15.90	$1.1e^{-6}$ ***	$7.1e^{5}$	17	6.63	$5.1e^{-10}$ ***	$4.0e^{5}$	34	1.87	0.010 **	$5.9e^{5}$	93
Wood Density	0.25	2	17.52	$5.3e^{-7}$ ***	0.55	11	7.00	$4.5e^{-8}$ ***	0.32	22	2.03	0.013 *	0.55	77
Huber Value	15.36	2	36.35	$2.5e^{-11}$ ***	2.95	9	1.55	0.149	5.71	18	1.50	0.118	13.73	65
Pith Ratio	26.22	2	39.28	$1.4e^{-12}$ ***	19.36	13	4.46	$1.2e^{-5}$ ***	8.85	26	1.02	0.454	26.36	79
Pith Percent	19.11	2	45.01	$8.9e^{-14}$ ***	11.77	13	4.26	$2.3e^{-5}$ ***	6.25	26	1.13	0.328	16.78	79

Model B

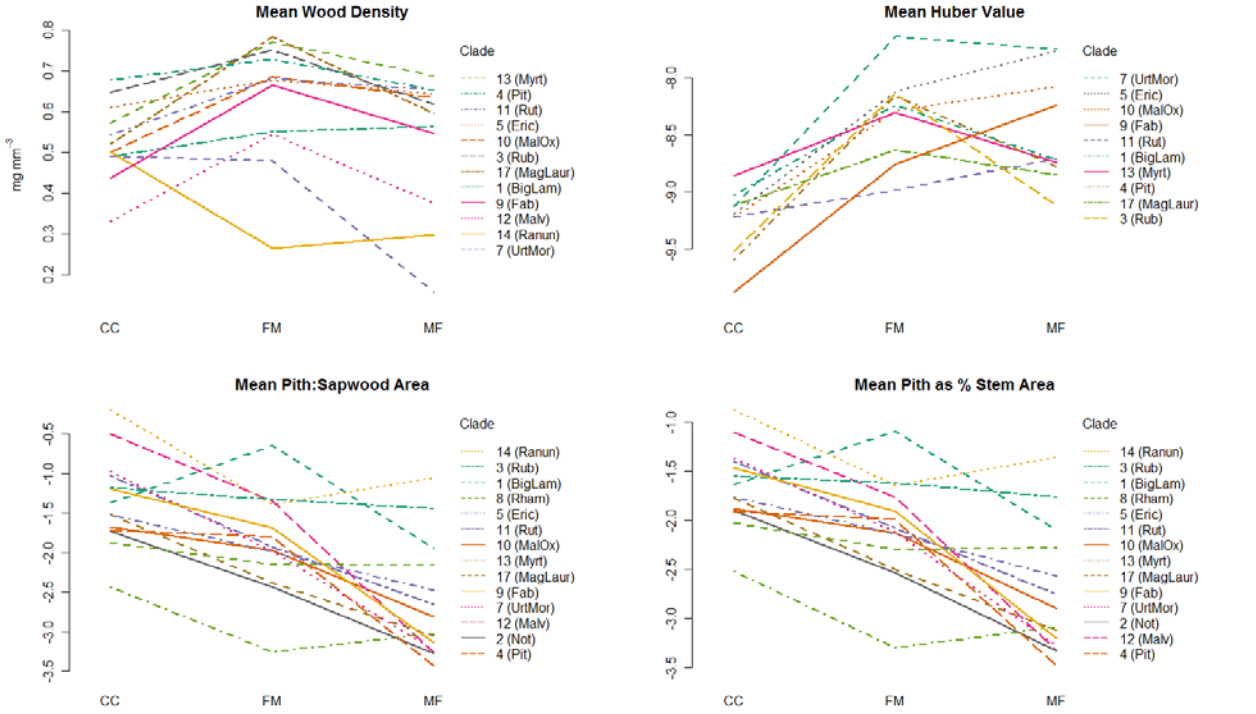
	Site				*Clade*								*Residuals*	
	Sum Sq	*df*	*F*	*p*	*Sum Sq*	*df*	*F*	*p*					*Sum Sq*	*df*
Leaf Length	7.76	2	10.36	0.001 **	5.72	8	1.91	0.129	-	-	-	-	5.99	16
Leaf Area (p)	33.07	2	15.25	$3.3e^{-5}$ ***	11.56	14	0.76	0.698	-	-	-	-	30.36	28
Leaf Area (np)	28.80	2	12.62	$1.2e^{-4}$ ***	9.99	14	0.63	0.821	-	-	-	-	31.93	28
SLA (p)	0.72	2	1.15	0.330	5.38	14	1.23	0.311	-	-	-	-	8.77	28
SLA (np)	1.01	2	1.57	0.225	5.81	14	1.30	0.270	-	-	-	-	8.97	2
LDMC (p)	$6.1e^{4}$	2	4.19	0.023 *	$4.5e^{5}$	17	3.62	$6.8e^{-4}$ ***	-	-	-	-	$2.5e^{5}$	34
LDMC (np)	$6.6e^{4}$	2	4.47	0.019 *	$4.0e^{5}$	17	3.23	0.002 **	-	-	-	-	$2.5e^{5}$	34
Wood Density	0.08	2	5.09	0.015 *	0.48	11	5.34	$4.2e^{-4}$ ***	-	-	-	-	0.18	22
Huber Value	5.22	2	21.35	$1.8e^{-5}$ ***	2.00	9	1.81	0.135	-	-	-	-	2.20	18
Pith Ratio	11.85	2	27.26	$3.6e^{-7}$ ***	10.65	13	3.77	0.002 **	-	-	-	-	5.65	26
Pith Percent	8.57	2	27.70	$3.6e^{-7}$ ***	7.12	13	3.54	0.003 **	-	-	-	-	4.02	26

Table 13: Conserved Index The results of two different methods are shown for calculating CI; for details, see Methods. For A, the number of taxa at each site are shown, to indicate relative unbalance. In B, clade means only are considered.

	A. *Taxon Means*						B. *Clade Means*			
	Taxa									
	Clades	*CC*	*FM*	*MF*	*CI*	*p*	*Clades*	*CI*	*p*	
Leaf Length	9	35	14	12	0.26	0.021	*	9	0.33	0.120	
Leaf Area (p)	15	65	34	26	0.12	0.022	*	15	–0.11	0.726	
Leaf Area (np)	15	64	34	26	0.07	0.141		15	–0.18	0.849	
SLA (p)	15	65	34	26	0.16	0.003	**	15	0.11	0.293	
SLA (np)	15	64	34	26	0.21	0.001	**	15	0.12	0.282	
LDMC (p)	18	79	37	30	0.37	< 0.001	***	18	0.52	< 0.001	***
LDMC (np)	18	80	37	30	0.35	< 0.001	***	18	0.47	< 0.001	***
Wood Density	12	67	25	21	0.38	< 0.001	***	12	0.69	< 0.001	***
Huber Value	10	56	20	19	0.04	0.215		10	0.24	0.151	
Pith Ratio	14	71	27	23	0.29	< 0.001	***	14	0.53	0.003	**
Pith Percent	14	71	27	23	0.27	< 0.001	***	14	0.51	0.003	**

Two methods of calculating CI were compared. Model A compared means for taxa within clades at each site, allowing the calculation of a site by clade interaction. Model B compares a single mean for each clade and site; the interaction is necessarily omitted. For all traits, clade explained more of the total variation observed under Model A than Model B (Table 12). Leaf dry matter content, wood density, and relative size of the pith were highly conserved between lineages (27–69%, $p \leq 0.003$). Leaf area and Huber value had a low CI, which was only weakly significant, or non-significant. In fact, under Model B, clade explained less variation in leaf area than would be expected to occur by random processes (resulting in a negative CI value). Interestingly, SLA was intermediate—perhaps because it is a function of leaf dry mass (highly conserved) normalized to leaf area (apparently labile.) It was significantly conserved in Model A (16–21%, $p \leq 0.003$), but not in Model B (11–12%, $p > 0.29$).

Figure 8: Site-by-clade interactions in wood traits Site codes are CC (Canopy Crane, wet tropical), FM (Forty Mile Scrub NP, dry tropical), and MF (Mt. Field NP, temperate). Clades are listed in order of occurrence at Mt Field. Parallel lines indicate trait conservatism; crossed lines indicate that a clade changes its position within the community.



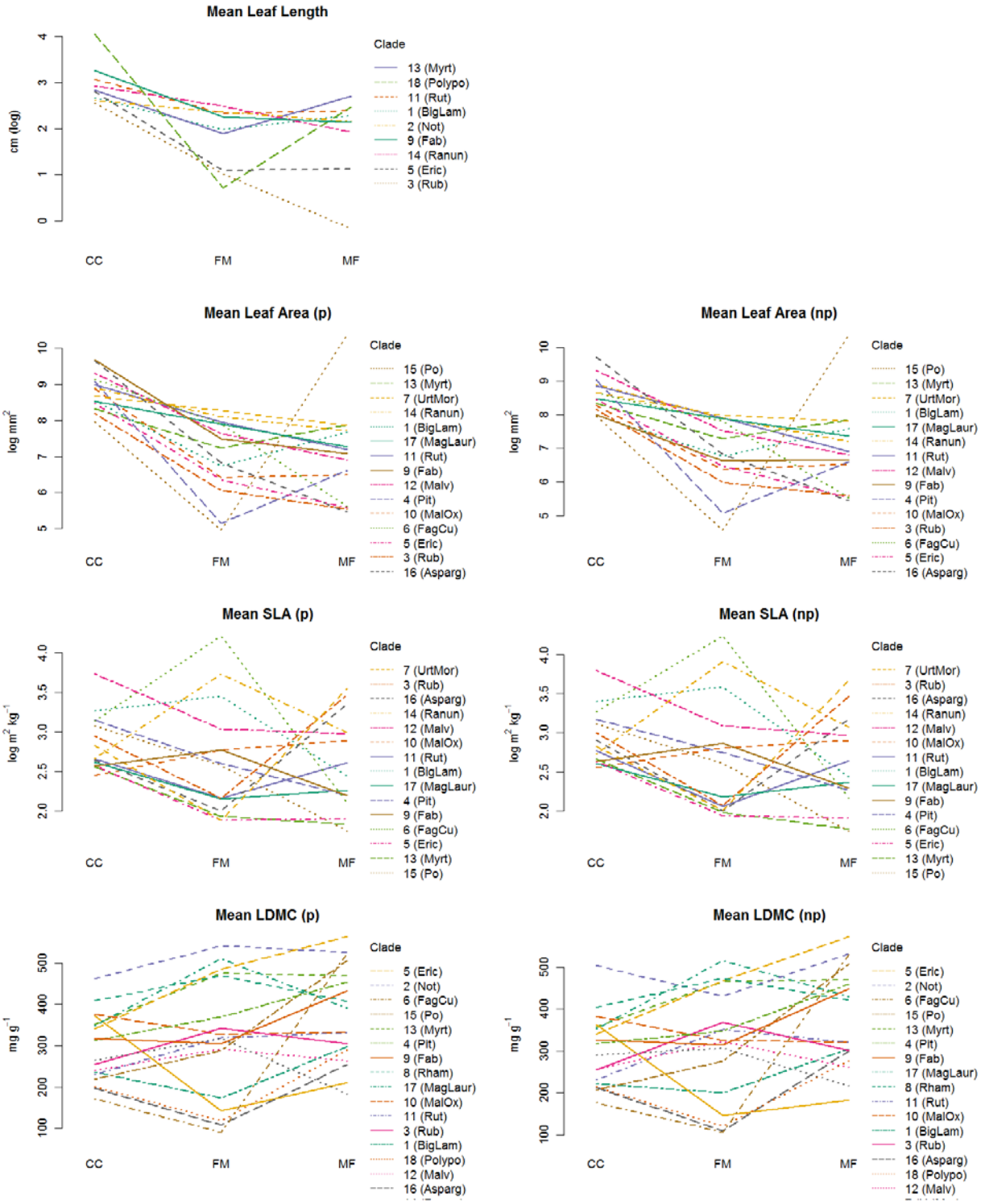


Figure 9: Site-by-clade interactions in leaf traits Site codes are CC (Canopy Crane, wet tropical), FM (Forty Mile Scrub NP, dry tropical), and MF (Mt. Field NP, temperate). Clades are listed in order of occurrence at Mt Field. Parallel lines indicate trait conservatism; crossed lines indicate that a clade changes its position within the community.

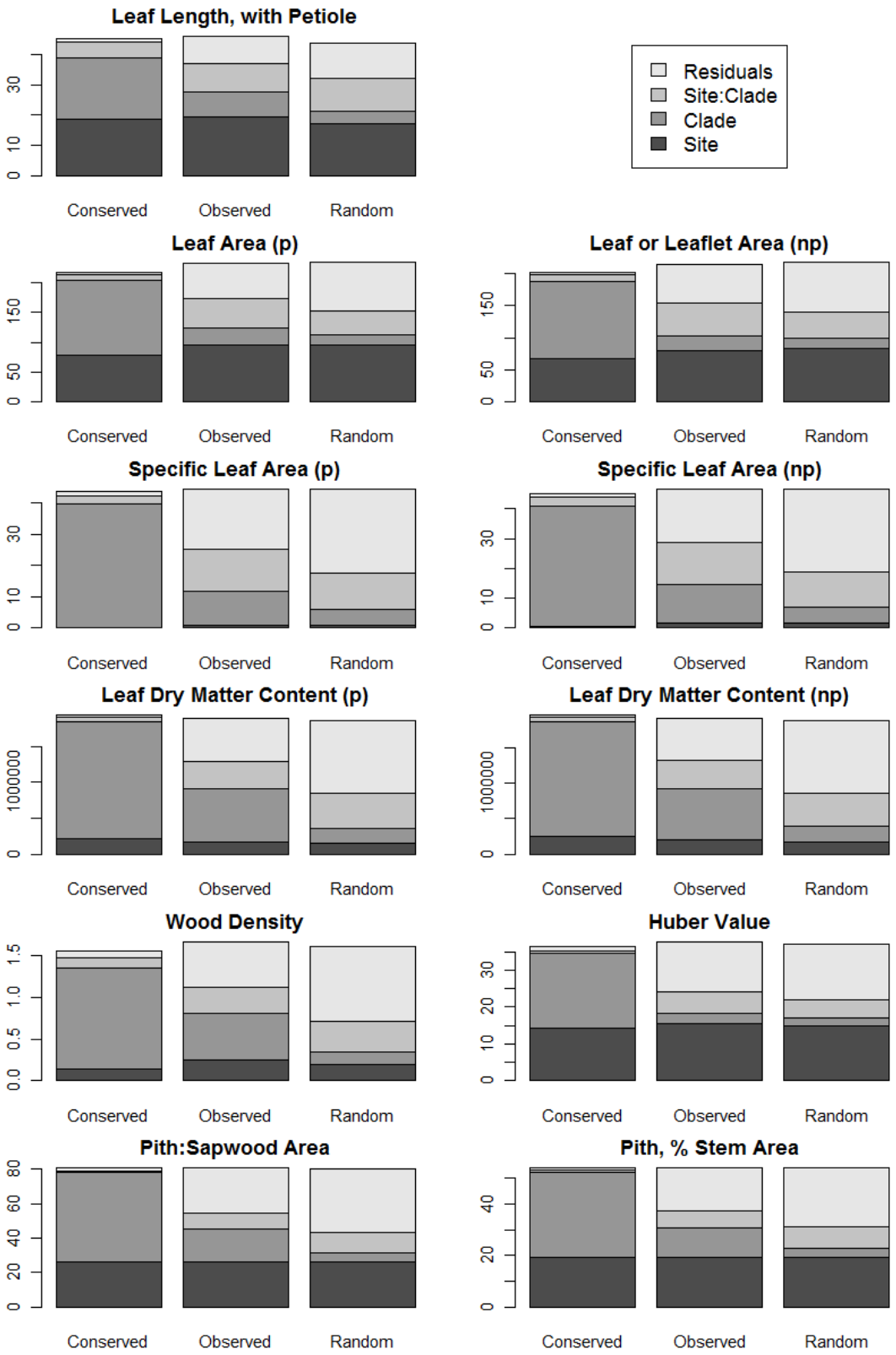


Figure 10: Effect of clade as a proportion of total variance (Model A: Taxon Means) Results compare the observed data (center, each graph) with a fully conserved model (left) and the mean of 100 randomized models (right.)

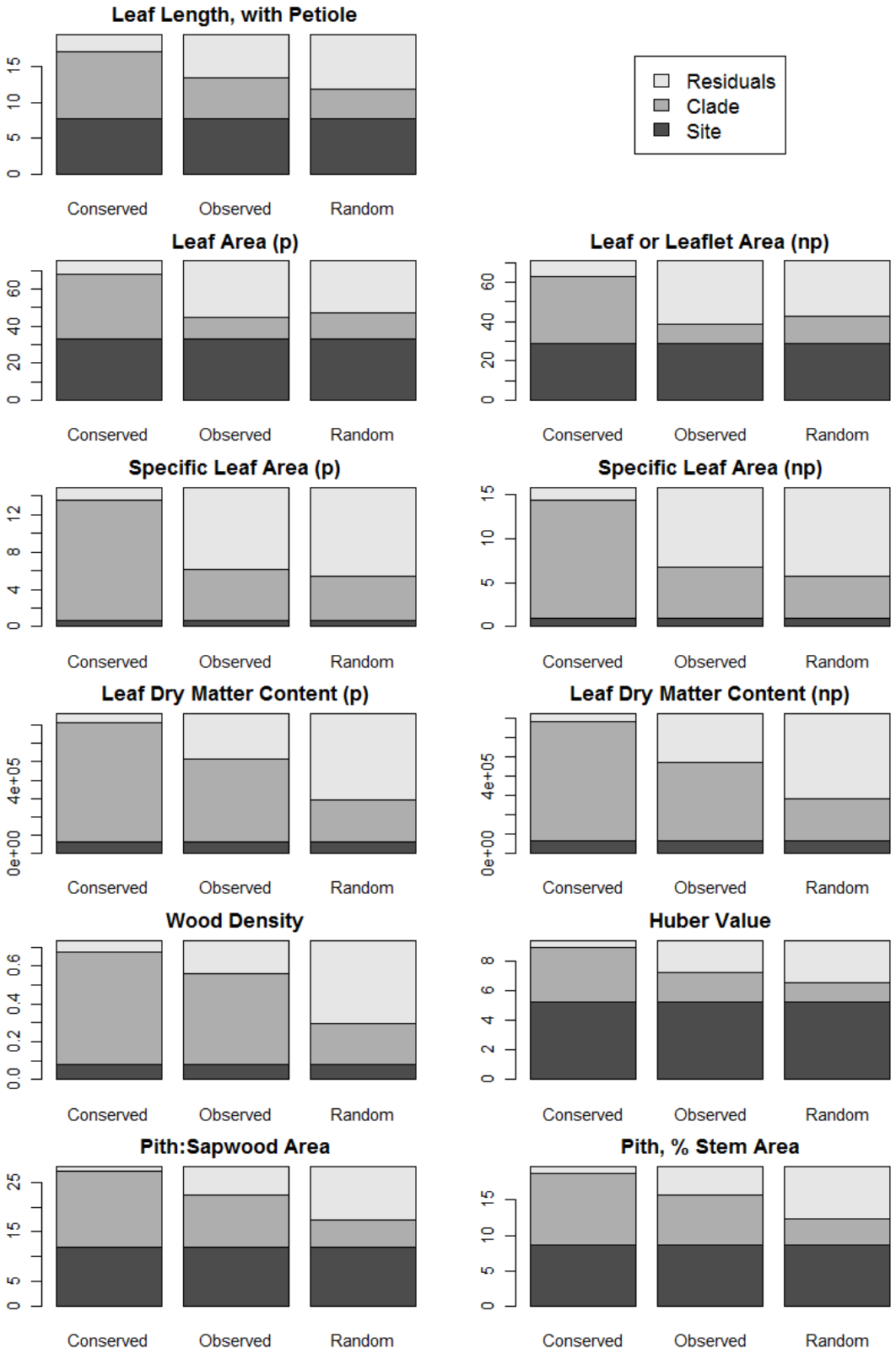


Figure 11: Effect of clade as a proportion of total variance (Model B: Clade Means) Results compare the observed data (center, each graph) with a fully conserved model (left) and the mean of 100 randomized models (right.)

Discussion

Functional trait variation increases in response to seasonality

Hypothesis 1 predicted that functional trait variance would be higher in seasonal environments, indicating a spectrum of coexisting functional strategies. Post-hoc tests consistently showed higher variance in all traits at the temperate site than at the warm tropical site. They also frequently linked variance patterns at the two seasonal sites, which were not statistically different in all but two tests. Patterns in means varied. The wet tropical and temperate site had significantly different means for most traits. In keeping with other literature, the dry tropical site showed a pattern that was intermediate between the wet tropical and temperate environments, with some traits matching temperate means, and others matching wet tropical means (ex., Wright *et al.* 2005; Moles *et al.* 2007, 2009, 2011; Chave *et al.* 2009). However, there were very few traits for which means at the dry tropical environment were distinct from both of the other two sites.

A corollary to Hypothesis 1 predicted that greater variance would correspond to tolerance of the most limiting conditions—drought and cold—in each environment. Leaf traits linked to drought tolerance, and therefore survival, include smaller leaf areas, higher specific leaf area, and higher leaf dry matter content. Smaller leaf areas have also been linked to cold tolerance, through the global latitudinal gradient in average leaf size (Moles *et al.* 2011). Cold, particularly freezing, and drought are closely linked at a physiological level; for living leaf tissue, surviving frost is essentially survival of intense, localized dehydration (Steponkus 1984; Uemura, Joseph, & Steponkus 1995; Thomashow 1999; Ball *et al.* 2002b). As a result, it is perhaps not surprising that smaller leaves were found at both of the seasonal sites, and that all three traits showed greater variance at the seasonal sites. This may indicate a spectrum of coexisting desiccation tolerance strategies.

Wood density has also been linked to surviving the low water potentials associated with drought (Hacke *et al.* 2001; Jacobsen *et al.* 2005), although vessel diameter is more closely correlated, and the two are not always linked (Chave *et al.* 2006; Preston, Cornwell, & DeNoyer 2006; Zanne *et al.* 2010). A peak in variance in wood density at the droughted site was predicted, due to the co-existence of drought-deciduous and evergreen trees, two alternative strategies for surviving the dry season. This was not supported. Although variance in wood density was highest in magnitude at the dry site, it was not significantly higher than at the other two sites. This may be due to the fact that while both strategies exist at the site, the dense-wooded strategy is far more common. Of the 22 taxa with wood density greater than 0.7 mg mm^{-3} in this study, 15 are found at the dry site, as compared with only 4 of 23 trees with wood density less than 0.4 mg mm^{-3}.

There were several other interesting patterns relating to the breadth of the trait spectrum under different seasonality conditions. Notably, SLA, a trait strongly linked to carbon capture and life history strategies (Reich, Walters, & Ellsworth 1997; Wright *et al.* 2004), showed no shift in mean between the three sites. Given the large sample size (n = 233), it is unlikely that this was due to a lack of statistical power. Instead, variance within each site is actually higher than variance among the sites. This suggests that each community has the full range of functional strategies implied by a large spread in SLA, from high-SLA pioneers to lower-SLA canopy dominants (Westoby 1998; Reich &

Oleksyn 2004; Wright *et al.* 2004, 2005). Nonetheless, there is a significantly larger range of coexisting SLA values at the two seasonal sites.

These results also bear on niche differentiation and species diversity. The wet tropical site is far more diverse than either of other two sites, and it draws on a much larger local species pool (Table 9). Yet there was consistently no evidence of greater trait variance at this site. Therefore, this study can add to evidence rejecting the hypothesis that higher functional diversity is a correlate of higher species richness (Schwilk & Ackerly 2005; Condit *et al.* 2006; Kraft, Valencia, & Ackerly 2008). Although this study does not distinguish between alternative possible mechanisms, such as narrower niche breadths or tighter niche packing, it can falsify the proposition that the tropics are more diverse because of constant niche breadth and a wider trait gradient.

Discussion

Both phylogenetic niche conservatism and local adaptation play a role in observed trait variance

The second aim of this study was to investigate the relative role of phylogenetic niche conservatism in shaping functional trait values under contrasting seasonality conditions. At the continental scales examined in this analysis, phylogenetic niche conservatism appears to explain a moderate, and frequently significant, amount of variation in trait values among related clades. The percent of variance explained by trait conservatism ranged from virtually none (Huber value, 4%) to as much as one third to one half (leaf dry matter content and wood density; Table 13.) Perhaps one of the surprising results of this analysis is that traits do not have to be very conserved to explain a significant proportion of variation. Comparing Figures 8 and 9 with Table 13 demonstrates that although the proportion of variation attributable to clade for each significantly conserved trait is greater than that observed by random, it is far less than would be expected if traits were perfectly conserved. This represents an important caution for research in this area: significant conservatism does not necessarily equal strong conservatism, particularly when compared to the maximum possible.

As predicted, traits relating to wood structure—wood density and relative pith area—were highly conserved. This suggests either that wood is less labile in response to environment, under less direct selective pressure, or species track habitat more strongly in relation to their wood traits. Wood density has been linked to both growth rate and successional status (Enquist *et al.* 1999; Muller-Landau 2004; King *et al.* 2006; Chave *et al.* 2009), as well as to mortality (Kraft *et al.* 2010). Pith percent has received less attention, but is linked to mechanical properties of the stem (Jacobs 1954; Cipollini 1999; Briand *et al.* 1999). The proportion of stem area that is made up by pith has clear implications for tradeoffs in mechanical strength and construction costs: for the same amount of material, a cylinder is much stronger than a rod; but for the same outer diameter, requiring far more material, a rod is stronger. As a result, pith characters may reflect the difference between fast growing, low-investment stems and slow-growing, high-investment stems. In this data set, both wood density and pith percent were significantly correlated with SLA ($R^2 = 0.202$, $p = 3.55e^{-10}$ and $R^2 = 0.203$, $p = 3.58e^{-10}$, respectively), now well established as an indicator of the spectrum between

these two strategies (Wright *et al.* 2004). Conservatism of these characters could indicate the tracking of particular successional strategies across environments.

Leaf area and Huber value (sapwood-to-leaf-area ratio) both showed no evidence of conservatism. As described above, leaf area has been linked to both cold and drought survival (Givnish 1987; Ackerly & Reich 1999; Ackerly *et al.* 2002; Moles *et al.* 2011). The negative CI values observed for leaf area using clade means (Model B, Table 13) suggests that it is highly labile. This indicates that leaf area changes in response to environment, mostly likely as the result of both plasticity and local adaptation. As a ratio between sapwood area and leaf area, Huber value is clearly affected by leaf sizes (Zimmermann 1978; Pickup, Westoby, & Basden 2005). There is also evidence that Huber value is highly plastic, and that it responds rapidly to drought conditions (Williams *et al.* 1997; Cavender-Bares & Holbrook 2001; Burgess, Pittermann, & Dawson 2006; Maseda & Fernández 2006; Carter & White 2009; Ambrose *et al.* 2010). Indeed, reduction in canopy area through leaf abscission is one of the most basic and well-documented responses to water stress (Levitt 1980). Both traits appear to change in response to their environment rather than tracking a particular environment.

The intermediate picture presented by the other leaf traits is also noteworthy. Surprisingly, SLA, which, like wood density, is linked to relative growth rate and competitive strategy (Westoby 1998; Wright *et al.* 2004) is weakly conserved, or not conserved at all. By contrast, LDMC is strongly conserved under both calculation models. This is interesting as the two are often thought to relate to similar aspects of carbon capture strategy (Ryser & Urbas 2000; Garnier *et al.* 2001). It has been reported that, within species, LDMC is more stable across larger spatial and temporal scales, while SLA is more stable at smaller temporal scales (Garnier *et al.* 2001). Wilson et al. (1999) also found less intraspecific variation in LDMC than SLA, suggesting less environmental plasticity. These results may indicate that, in the communities studied here, LDMC tracks habitat more tightly than SLA, or that SLA responds, either through adaptation or plasticity, to local conditions more quickly. Nonetheless, other studies have found significant conservatism in SLA (Ackerly 2003, 2004). That these two traits may track different environmental and evolutionary signals is an interesting direction for future research.

Conclusions

Given the large scale geographical scale this analysis (~3,000 km) perhaps it is not surprising that patterns observed show a blend of local adaptation and phylogenetic niche conservatism. Indeed, there were few close divergences that spanned all three sites. The clades examined most closely correspond to the traditional taxonomic categories of families, orders, or even superorders, and most are estimated to have diverged from one another between 50 and 130 Ma. This pattern could suggest a trailing-edge scenario (sensu Ackerly 2003), in which the notophyll forest communities have slowly adapted to seasonal conditions, or an environmental island scenario (also sensu Ackerly 2003), in which pockets of notophyll forest are maintained in the absence of stronger competitors, perhaps due to the isolation of the Australian flora due to long dispersal distances from other major floristic centers.

At the same time, shifts in variance between the communities examined suggest that climatic pressures can cause divergent selection. The pattern of greater variance at the two seasonal sites gives credence to this. This could be due, in part, to unrelated taxa—the majority of taxa in each community do not have close relatives at other sites, and therefore are not included in the clade-based analysis presented here. These taxa may account for a disproportionately large amount of the variance between sites. It could also be due to divergent selection on related taxa within the seasonal communities—clades with relatively high values are pushed higher, while clades with relatively low values are pushed lower.

The findings of this study support those of Chapter 1, which demonstrated that the high taxonomic diversity of tropical communities is likely a result of their age, rather than an intrinsically higher speciation rate. In keeping with that finding, tropical taxonomic diversity also does not correspond to a high functional diversity; instead, higher functional diversity is associated with seasonal environments. The greater variety of environmental conditions found in seasonal environments may be more likely to exert divergent selective pressures on the taxa found there. In particular, the finding of greater functional variance in seasonal environments suggests a mechanism for the higher diversification rates observed in clades that cross climatic boundaries.

Chapter 3: Freezing under contrasting seasonality: Dry season survival as a stepping stone to cold tolerance

Introduction

Flowering plants have spent most of their evolutionary history in tropical environments (Wing & Boucher 1998; Willis & McElwain 2002). To this day, angiosperms are most diverse in tropical regions, suggesting that tolerance to seasonal cold does not evolve easily in this clade (Woodward 1987; Wiens & Donoghue 2004, see also Chapter 1.) Nonetheless, flowering plants form a major part of most temperate ecosystems, and it is evident that tropical to temperate shifts have been repeated many times. How did the various tropical ancestors of today's temperate angiosperms adapt to conditions that were potentially lethal? As sessile, exothermic organisms, plants have a limited ability to avoid unfavorable conditions; any long-lived plant species in a temperate climate must find a way to survive winter.

As with many major evolutionary changes, there is a potential stepping stone in the adaptive landscape (Gould & Vrba 1982). Seasonal drought may provide a link between the tropical origins and present temperate diversity of flowering plants. Studies at both the whole-plant and cellular levels show strong connections between surviving drought and surviving freezing (e.g., Mantyla, Lang, & Palva 1995; Close 1997; Ewers *et al.* 2003; Cavender-Bares *et al.* 2005; Medeiros & Pockman 2011). Today, many parts of the tropics experience significant dry seasons, driven by the global convective cycles, ocean currents, and rain shadow effects. During the late Cretaceous and early Tertiary, when many modern angiosperm groups diversified (Magallón & Sanderson 2001; Magallón & Castillo 2009), there is reason to believe that these same mechanisms operated. Although at a global level, this period was far warmer and probably much wetter than the present, both mineral formations and leaf physiognomy give evidence of local aridity at multiple points in space and time (Hallam 1984; Zachos, Stott, & Lohmann 1994; Price, Valdes, & Sellwood 1997; Wilf *et al.* 1998; Bolle *et al.* 1999; Wilf 2000; Zachos *et al.* 2001; Jenkyns 2003; Wing *et al.* 2005; Moriya 2011).

Linking drought and freezing

There are many ways plants can be killed by low temperatures (Levitt 1980; Sakai & Larcher 1987; Pearce 2001); however, research has focused primarily on two targets: xylem embolism and damage to living tissue. Xylem embolism is an important mechanism of freeze damage, and has been shown to set the range limits for a variety of different species (e.g., Sperry & Sullivan 1992; Langan, Ewers, & Davis 1997; Davis, Sperry, & Hacke 1999; Pockman & Sperry 2000; Cavender-Bares & Holbrook 2001; Stuart *et al.* 2007)(e.g., Sperry & Sullivan 1992; Pockman & Sperry 1996; Langan, Ewers, & Davis 1997; Davis, Sperry, & Hacke 1999; Cavender-Bares & Holbrook 2001; Stuart *et al.* 2007). However, this chapter focuses exclusively on potential damage to living tissue. Acclimation to both freezing and drought appears to share common pathways of signaling, transcription and proteome changes (Close 1997; Thomashow 1999; Hara 2010). By contrast, a variety of evidence suggests that xylem embolism formation due to drought and freezing occur by very different mechanisms (Sperry & Tyree 1988; Tyree & Sperry 1989; Sperry *et al.* 1994; Davis, Sperry, & Hacke 1999).

There are strong links between survival of drought and freezing at the cellular level (Close 1997; Thomashow 1999; Xin & Browse 2000). In living tissue, damage caused by both processes is surprisingly similar: the primary mechanism is membrane deformation due to dehydration (Henckel 1964; Steponkus 1984; Steponkus, Uemura, & Webb 1993; Thomashow 1999). Either low water potentials, or the formation of ice in extracellular spaces, can provide a strong gradient drawing water out of cells (Ball *et al.* 2002b, 2006; Roden *et al.* 2009). The loss of hydration shells around phospholipid membranes can lead to lysis, through deformation, lesions, or phase transformations (Steponkus 1984; Steponkus, Uemura, & Webb 1993; Uemura, Joseph, & Steponkus 1995; Uemura & Steponkus 1997).

In order to resist freezing, living plant tissue requires two things: prior seasonal exposure to induce acclimation, and the adaptive ability to acclimate. The ability to survive freezing is not constitutive; instead, plants must be exposed to low, but not freezing temperatures (~6–2°C, Xin & Browse 2000). After exposure, many plants show greatly increased tolerance to, and ability to recover from, freezing stress (Levitt 1980; Thomashow 1999; Xin & Browse 2000). However, even with such exposure, not all plants can acclimate to freezing. The ability to acclimate varies greatly among different groups of plants, and this variation is generally considered adaptive variation (Rehfeldt 1986; Allen & Ort 2001; Stinchcombe *et al.* 2004; Hannah *et al.* 2006; Zhen & Ungerer 2008). Multiple links between drought and freezing acclimation have been demonstrated in studies using genomic and proteomic techniques, as well as in whole-plant studies (Siminovitch & Cloutier 1982; Nordin, Heino, & Palva 1991; Lång & Palva 1992; Thomas & James 1993; Yamaguchi-Shinozaki & Shinozaki 1994; Baker, Wilhelm, & Thomashow 1994; Wang *et al.* 1995; Medeiros & Pockman 2011; Kosova *et al.* 2011). This effect is also recognized in horticultural and forestry literature, which often suggests acclimating seeds or seedlings by exposure to drought before planting when there is danger of frost (e.g., Waisel 1962; Evenari 1964; Costa e Silva *et al.* 2009; Coopman *et al.* 2010).

Research into shared modes of acclimation to freezing and drought focuses primarily on the accumulation of a related family of proteins, known as dehydrins or late-embryogenesis abundant group 2 (LEA–2) proteins (Close 1997; Thomashow 1999; Hara 2010). These genes are regulated by both water deficit and low temperatures (Thomashow 1999; Bray 2004). Transcription and translation produce small, intrinsically unordered proteins with both drought and cryoprotective function (Cheng *et al.* 2002; Hara *et al.* 2003; Chandra Babu *et al.* 2004; Puhakainen *et al.* 2004; Houde *et al.* 2004; Yin *et al.* 2006; Brini *et al.* 2007). Although dehydrin function is an area of ongoing investigation, research has elucidated several possible mechanisms. Dehydrins are highly hydrophilic, and many show the ability to bind to a variety of large and small cellular structures, including enzymes, membranes, and cytoskeleton. As such, they are thought to act as chaperones, stabilizing these structures in the absence of water (Dure *et al.* 1989; Close 1997; Hara 2010).

In the design of this study I faced a choice: whether to examine tolerance to freezing in acclimated or unacclimated tissues. A number of previous studies have been successful in inducing acclimation in samples after harvesting (e.g., Pogosyan & Sakai 1969; Sakai 1970; Sakai & Weiser 1973). Value has also been placed on studying unacclimated tissue as the most direct way to observe the

mechanisms of cold damage to the tissues themselves (Allen & Ort 2001). Given the evolutionary motivation behind this chapter, i.e., whether previous experience of drought could provide a prophylactic effect against unanticipated frost, I chose to work with unacclimated tissue. I believe this most closely mimics the conditions that are of interest—whether an unaccustomed tropical plant that is adapted to seasonal drought can leverage this adaptation to survive seasonal cold.

Tropical to temperate transitions in the past and present

The goal of this study is to explore how climatic regime affects freezing damage in a natural system, by looking for evolutionary connections between close relatives. To explore the effects of seasonality in field collected plants, three sites with contrasting seasonality conditions were selected (Figure 12): wet tropical ("warm and wet," abbreviated WW,) seasonally-dry tropical ("warm and dry," abbreviated WD,) and temperate with a high rainfall ("cold and wet," abbreviated CW.) Although all three communities thrive today, they also reflect relevant past conditions. The majority of Australia was covered in wet tropical rainforest throughout the late Cretaceous and early Tertiary (~103–44 Ma, Christophel & Greenwood 1989). However, there were also locally arid areas at this time (Martin 2006), and there is evidence that another type of vegetation may have existed: monsoonal forest or vine thicket (Greenwood 1996). Monsoonal forest shares strong physiognomic and phylogenetic links with wet tropical rainforest, but is found in areas with pronounced wet and dry seasons (Webb 1959, 1968; Specht 1981; Webb & Tracey 1981). During the early Oligocene (~35–34 Ma), Australia experienced dramatic cooling and drying trends when the southern edge of the continent separated from Antarctica (Christophel & Greenwood 1989; Zachos, Quinn, & Salamy 1996; Zachos *et al.* 2001; Martin 2006). With the appearance of seasonally cold temperatures for the first time in ~50 million years, a third type of vegetation was established: cool-temperate rainforest. The first evidence of plants adapted to seasonal cold appears in the Australian paleobotanical record in the Oligocene (Kemp 1978; Hill 1984; Macphail *et al.* 1991).

Relatively few studies have examined frost tolerance in both tropical and temperate plants. Fewer still have compared related species pairs. Read and Hope (1989) compared the freeze tolerance of *Nothofagus* from tropical and temperate collection sites in Australia and New Guinea, with the goal of understanding the present-day and paleobotanical distribution of this genus. Bannister and Lord (2006) compared the frost tolerance of Southern Hemisphere plants from four continents, grown in a common botanical garden setting. They found them to be more frost tolerant than predicted by climatic regime in the collection locality; however, they also found that frost response to be more closely linked to provenance than to family or genus. Cunningham and Read compared growth (2003) and photosynthetic response (2002) in plants collected from tropical, subtropical and temperate environments in eastern Australia and grown in common gardens at several different temperature regimes. They found evidence that cool, non-freezing temperatures limited both carbon assimilation and growth rate in the tropical-collected species as compared with temperate collections. However, their approach did not include related pairs from different provenances. Sakai (1970) explored freezing resistance in topical and temperate willow species, and Sakai and Weiser (1973) studied a number of pines, among other taxa, from across latitudinal gradients in North

America, using excised material acclimated in the lab. Both studies showed greater tissue death in plants from warmer, less seasonal collection localities.

In order to investigate whether seasonal drought could protect taxa with different evolutionary histories against frost, this study examines seven widespread clades. In each clade, work focuses on the minimum monophyletic unit found at all three sites (Table 15). The goal of this project is to test two hypotheses. The first, based on a possible physiological analog of the global change from warm to seasonal and cold that took place during the evolution of angiosperms, is: 1) Plants from the dry tropical site will show greater tolerance to freezing than plants collected from the wet tropical site, in spite of the fact that they have not been acclimated to freezing temperatures. The aim of this is not to recreate past shifts, as the past adaptive environments of the clades studied are still unclear. Rather, it is to explore whether the established link between drought and freezing is a plausible mechanism for transitions between dry tropical and temperate environments. Second, this study also explores whether there is evidence that some related groups have a greater ability to survive shifts between warm to cold. As discussed above, there is evidence of genetic variation in the ability to acclimate to cold, and it is widely believed that some clades of plants show less ability than others to evolve cold acclimation (Rehfeldt 1986; Takhtajan, Crovello, & Cronquist 1986; Allen & Ort 2001; Stinchcombe *et al.* 2004; Hannah *et al.* 2006; Zhen & Ungerer 2008). This leads to the second hypothesis: 2) There will be phylogenetic trends in tolerance to cold—some clades will be more resistant to cold than others, independent of the conditions from which they were collected. However, no *a priori* prediction is made as to which of the seven clades will show greater patterns of frost tolerance.

Methods

Site selection

Australian notophyll forests are an excellent system for studying plant communities under differing conditions of seasonality. These communities occur across a wide latitudinal gradient, from Tasmania (43°S) to northern Queensland (10°S), and into Papua New Guinea (±0°S). Three sites where this vegetation occurs in protected areas were chosen: a temperate site at Mt. Field National Park in Tasmania, a dry tropical site at Forty Mile Scrub National Park in Queensland, and a wet tropical site at the Australian Canopy Crane Research Station in Daintree National Park, also in Queensland. There is a history of ecological research at all three sites, and long-term plot surveys are available for the two sites in Queensland.

Local temperate records from at least three weather stations near each site demonstrate that differences in temperature and rainfall exemplify the contrasts between wet tropical, dry tropical, and wet and cold this study aims to test (Australian Government Bureau of Meteorology 2011; Mark Twist, Undara Experience, pers. com.) While all three communities experience some seasonality, the total amount of rainfall at the wet tropical site ensures a consistent supply of water year-round. Although the dry tropical site experiences colder winter minimums than the wet tropical site, these are well above freezing, and highs consistently exceed those at the wet tropical site (Figure 12). This results in an overlapping temperature range between the two warm sites. The lowest extreme

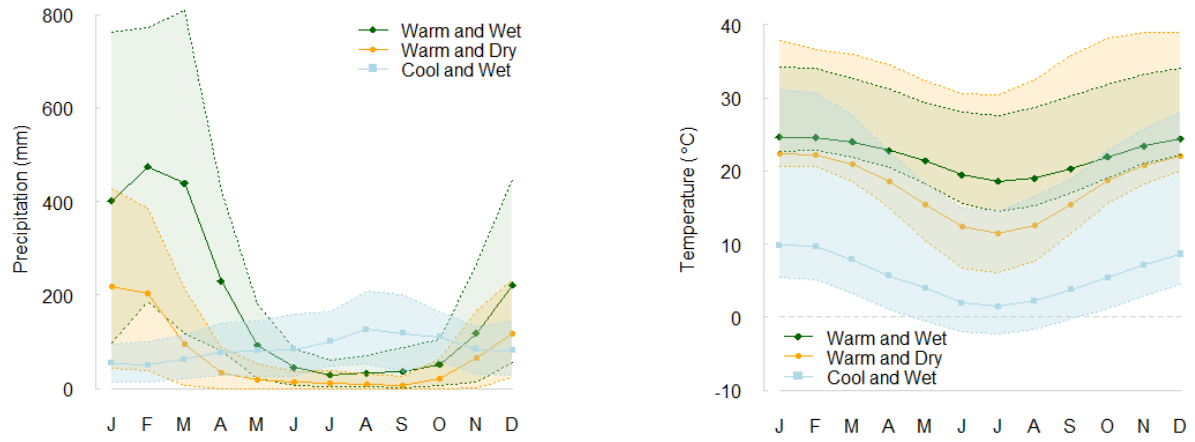


Figure 12: Rainfall and daily temperature range at study sites (Repeated from Chapter 2) Solid lines with symbols show monthly means (precipitation) and monthly mean minimum (temperature) for the period 1970–2010. At left, shaded area and dotted lines show the 10th and 90th percentiles for monthly rainfall over the same period. At right, the shaded area shows the difference in daily temperature range, represented as the difference between the 10th percentile of minimum temperature, and the 90th percentile of maximum temperature, for each month over 40 years. Note the overlap of daily temperature range at both warm sites. Each figure is the average of the three closet weather stations in comparable climate zones to the study site. Although a standard data range of 1970–2010 was used for all sites, not all sites have continuous data during this period. In some cases, two nearby sites were combined (for example, a post office site that was closed in the 1990s was combined with an airport site for the same township opened in the 2000s.)

temperature experienced at the dry tropical site in the month leading up to sample collection was 13.6°C (Table 14), well above temperatures thought to induce frost acclimation (Xin & Browse 2000). It is also unlikely that cold acclimation could persist given the high winter maxima experienced at this site: deacclimation can occur rapidly in response to warmer temperatures (Barker *et al.* 2005; Loveys, Egerton, & Ball 2006; Kalberer, Wisniewski, & Arora 2006), and repeated cycles of warming can cause deacclimation in vegetation at large scales (Woldendorp *et al.* 2007; Gu *et al.* 2008). By contrast, conditions at the temperate site indicate that plants collected there would have had ample opportunity to acclimate to freezing. Local temperature records indicate 21 nights with freezing conditions in the 30 days before sample collection, with an overall low of ~–4°C (Australian Government Bureau of Meteorology 2011).

Taxon selection

Species lists for all three national parks were downloaded from government websites (Queensland Department of Environment and Resource Management 2009; Parks & Wildlife Tasmania 2010) and screened for genera and families found in all three parks. The resulting groups were then compared to concurrent surveys of the notophyll community at each site (Chapter 2), as well as to floristic resources (Curtis &

Table 14: Collection conditions Dates, absolute lowest and highest temperatures, and total precipitation recorded at nearby weather stations for each of the three sites in the 30 days before sample collection. Note that July and August are midwinter in the Southern Hemisphere.

	Wet tropical	*Dry tropical*	*Wet temperate*
Date collected	15- Jul-2010	30-Jul-2010	12-Aug-2010
Lowest temperature in month before collection (°C)	17.4	13.6	–4.3
Highest temperature in month before collection (°C)	29.1	32.8	17.4
Total rainfall in month before collection (mm)	24.5	0.0	84.1

Table 15: Taxa used in this study Letters to the left of each taxon name give the growth habit for each: e, canopy emergent; m, midstory, and u, understory.

Family	*Minimum taxonomic unit*	*Wet tropical*		*Dry tropical*		*Wet temperate*	
Fabaceae	subgenus *Phyllodineae*	*Acacia celsa*	m	*Acacia disparrima*	e	*Acacia verniciflua*	m
Myrtaceae	family	*Syzygium sayeri*	e	*Gossia bidwillii*	m	*Eucalyptus regnans*	e
Oleaceae	genus *Notelaea*	*Notelaea sp. (Cape York)*	m	*Notelaea microcarpa*	e	*Notelaea ligustrina*	u
Pittosporaceae	genus *Pittosporum*	*Pittosporum rubiginosum*	u	*Pittosporum spinescens*	m	*Pittosporum bicolor*	m
Rhamnaceae	family	*Emmenosperma cunninghamii*	e	*Alphitonia excelsa*	m	*Pomaderris apetela*	m
Rubiaceae	family	*Ixora biflora*	u	*Everistia vacciniifolia*	u	*Coprosma quadrifida*	u
Rutaceae	family	*Brombya platynema*	m	*Geijera salicifolia*	e	*Nematolepis squamea*	m

Morris 1956; Cooper & Cooper 2004; CSIRO 2010; Jordan 2011) to establish which members of each clade were considered members of the notophyll vegetation type. Taxon selection was also limited to woody taxa. Where several clade members occurred at a given site, the taxon that was most abundant in the community survey was preferred.

This resulted in the selection of seven clades for study. Three clades are at the generic level (Fabaceae: *Acacia*, Pittosporaceae: *Pittosporum*, Oleaceae: *Notealea*) and four are at the family level (Myrtaceae, Rutaceae, Rubiaceae, Rhamnaceae.) Family-level clades were used where a single genus did not occur at all three sites. All seven clades are eudicots; although magnoliid taxa were found at all three sites, no three were within a single family. Three groups are members of the larger rosid clade (Fabaceae, Myrtaceae, and Rutaceae,) and four are members of the asterid clade (Oleaceae, Pittosporaceae, Rhamnaceae, and Rubiaceae.) Taxa are listed in Table 15; throughout this chapter, clades will be referred to by their family names for convenience.

Assay of sensitivity to freezing

The ratio of variable to maximum chlorophyll florescence (F_v/F_m) is a measure of the functional status of photosystem II which has been widely used in plant ecophysiology (Ball *et al.* 1995; Maxwell & Johnson 2000; Cavender-Bares *et al.* 2004). Low temperatures reduce the rate of electron transport and therefore CO_2 fixation, due to improper electron transfer from the light-dependent to the light-independent reaction centers. This in turn leads to the production of reactive oxygen species and subsequent damage to functional center of photosystem II, as well as the thylakoid membrane itself (Smillie 1979; Öquist & Ögren 1985; Öquist, Hurry, & Huner 1993). This experiment used F_v/F_m as a simple, efficient assay of membrane damage caused by freezing. The technique has been used in many previous studies, and has become popular for its reliability and ease of use (Smillie & Hetherington 1983; MacRae, Hardacre, & Ferguson 1986; Smillie *et al.* 1987; Öquist & Huner 1991; Lindgren & Hällgren 1993; Roden & Ball 1996; Clement & van Hasselt 1996; Herzog & Olszewski 1998; Roden, Egerton, & Ball 1999; Rizza *et al.* 2001; Matsubara *et al.* 2002; Loveys, Egerton, & Ball 2006). Not only is F_v/F_m a direct measure of the functional status of photosystem II, it is also commonly strongly correlated with other methods of measuring membrane damage (Kamps *et al.* 1987; Öquist, Hurry, & Huner 1993; Herzog & Olszewski 1998; Loveys, Egerton, & Ball 2006; Ehlert & Hincha 2008).

However, it should also be noted that F_v/F_m is sensitive to a wide variety of environmental stresses, including drought and chilling as well as freezing (Bolhàr-Nordenkampf *et al.* 1989; Ball *et al.* 1995; Cavender-Bares *et al.* 2004). By design, one of the collection environments had previously experienced drought. In addition, the nature of the study required collecting leaves in the field and transporting them back to the lab to be frozen in a controlled environment. It is widely agreed that leaf tissue should be transported and stored at cool temperatures due to the risk of rotting (Cornelissen *et al.* 2003). Both of these conditions, however, mean that leaves may have been subjected to some form of mild stress before beginning the experiment. To account for this, statistical analyses were carried out relative decrease in F_v/F_m from the baseline measurement taken before the start of each experiment. For comparison, the first section of the results presents absolute F_v/F_m response along with the relative loss.

Sample collection

With three exceptions, five individuals of each clade were sampled at each site. The Cape Tribulation section of Daintree National Park is extremely large (~170 km^2) with few roads, and the forest is highly diverse, making it difficult to locate rarer species. One species, *Acacia celsa,* was collected at a site located within 10 km of the other collections; samples were taken from five different individuals. In the case of *Notealea* sp. (Cape York) and *Emmenosperma cunninghamii*, only a single individual of each species was located. The *Emmenosperma cunninghamii* individual was located within the Canopy Crane plot. Although *Notealea* is reported from Daintree National Park (CHAH 2010), no individuals were located at the listed locality. As an alternative, an undescribed tropical *Notealea* propagated from a parent plant in Cape York (original locality 12.6300°S, 143.4246°E; Chillie Beach, near Iron Range National Park; G. Sankowsky, pers. com.) was sampled from a private collection in Tolga, Queensland (163 km from the original site.) For *Emmenosperma*, five twigs were collected, and each was measured separately, but the mean of these measurements is reported as the measurement for a single individual. A single sample of *Notealea* sp. (Cape York) was collected and measured. Both clades were excluded from statistical analyses, and where shown in figures are explicitly labeled, so the reader may visually compare their responses to that of other clades.

At each site, leaf, or leafy twig, samples were collected from a portion of the canopy growing in full sun, or in the case of understory species, in the highest-light environment available (see Table 15 for a list of understory, midstory, and canopy species.) Samples were collected into polyethylene plastic bags lined with a damp paper towel. Bags were stored in an insulated cooler with a chilled, but not frozen, blue ice pack. A thin piece of Styrofoam was placed between the cool pack and the samples to avoid direct contact. The samples were transported in the cooler by air to the lab in Canberra, Australia on the following day, and stored in cool storage (4°C) for 1–3 nights before measuring. Equipment constraints limited measurements to no more than three species in a single day. However, ANOVA showed no significant effect of starting day on the initial fluorescence response for any site ($p = 0.292$).

Freezing protocol

Nadir temperature—the lowest temperature to be reached during a freezing profile—is an important choice in freezing assays. Lethal temperature varies greatly even among acclimated plants (Levitt 1980; Sakai & Larcher 1987). A minimum temperature of −10°C was chosen for two reasons. First, −10°C is sufficiently low to induce major cellular dehydration; over 90% of osmotically active water is believed to leave the cell at this temperature (Thomashow 1990). Second, the lowest temperature recorded near the cold site is −6.7°C, and it was desirable to bracket this degree of stress.

For each species, a baseline measurement was taken before beginning measurements by removing one leaf from each individual from cool storage and dark adapting for 30 minutes before measuring chlorophyll florescence (F_v/F_m) using a plant efficiency analyzer (Hansatech, King's Lynn, UK). From one or more remaining leaves, 11 samples were taken from each individual. An 8-mm diameter leaf punch was used to take samples from as few leaves as possible, with the same proportion of samples chosen from each leaf. For larger leaves, the midrib was uniformly avoided. For mid-sized leaves, in which the breadth of the leaf was close to the diameter of the borer, the midrib was uniformly included in every sample. The diameter of the leaf punch was such that undamaged, uncut tissue filled the entire measuring window of fluorescence meter clips. For species with leaves smaller that the diameter of the punch, an entire leaf was selected.

Each of the 11 samples was assigned to a nadir temperature between −1 and −10°C, or a positive control treatment, in which the punch was treated exactly as the other samples, but placed in cool storage at 4°C rather than frozen. It was removed from cool storage and measured along with the last sample removed from the water bath, to account for any damage due to the age of the sample, rather than the freezing treatment.

The freezing treatment was conducting using two programmable circulating water baths with chiller units (Julabo Labortechnik, Seelbach, Germany) filled with 1060g/L ethylene glycol in a 60:40 dilution, proving a circulating freezing point of ~−40°C. Sample disks were placed in 10 mL test tubes, which were partly submerged in the chilling baths. All disks were held at 4°C for 30 min, then cooled to −1°C over 30 min, then incubated at −1°C for a further 30 min. A small ball of ice was then placed in each test tube, in contact with the edge of the sample, in order to ensure ice nucleation. After a further 30 min incubation, one sample for each individual of each species was removed. From that point, the water bath was cooled one degree over thirty minutes, and held at each nadir temperature for 30 minutes before sampling. This was repeated to the nadir temperature of −10°C. Upon removing each set of samples, they were allowed to stand on the benchtop for 10 minutes to ensure thawing. After thawing, each sample was then removed from its test tube, and placed in a leaf clip. Samples were dark-adapted in the clips for 30 min at 4°C. Chlorophyll florescence (F_v/F_m) was then measured using the plant efficiency analyzer.

Statistical analysis

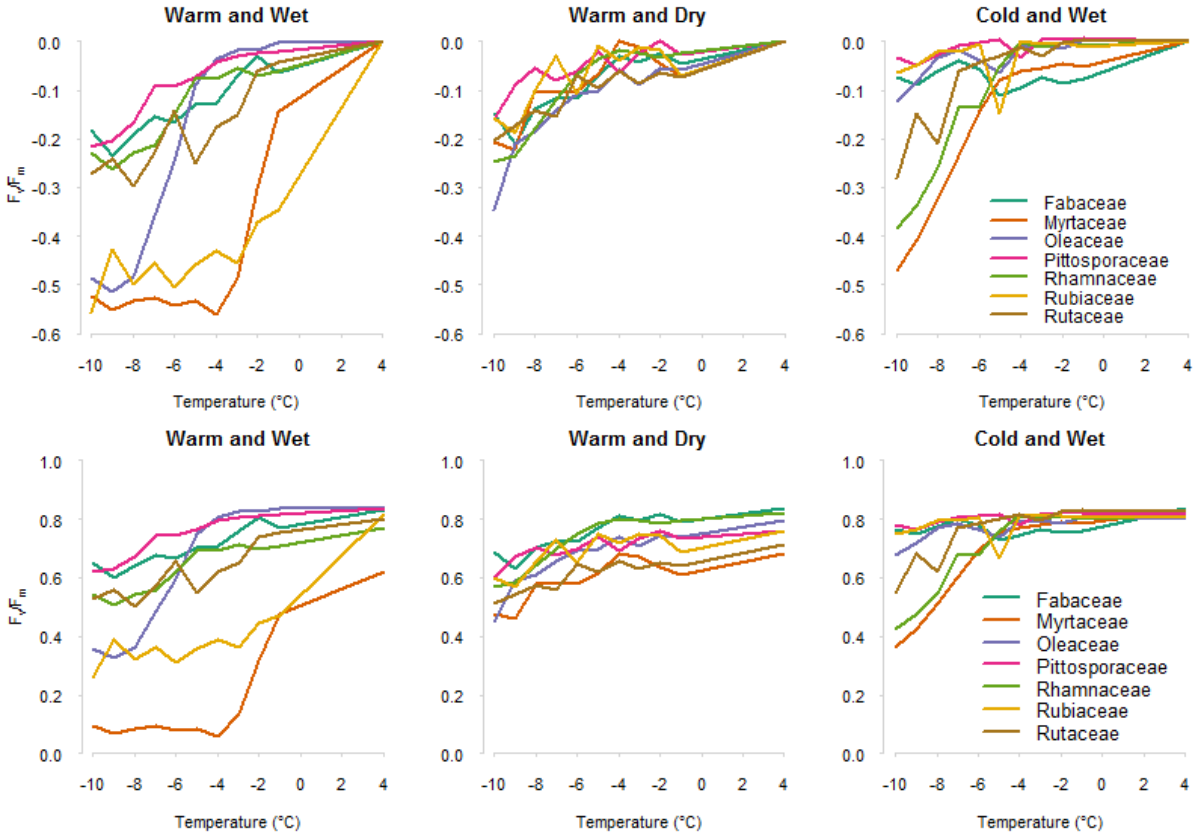
Three *a priori* comparisons were planned, based on questions of interest to the study: 1) Is the response to freezing different in plants from each of the three seasonality environments? 2) Does the response within each clade differ by seasonality environment? 3) Are clades different from one

another at different sites and temperatures? Three separate analyses of variance were performed to answer each of these questions. Complete ANOVA models for the full data set were explored, but due to complexities of nested individuals within species, and longitudinal measurement of non-linearly decreasing temperature, the appropriate experiment-wide model was difficult to construct and interpret. This approach is less powerful than using a single model; however, with the appropriate use of clade and site means, it is not pseudoreplicated. In all cases, ANOVA was followed by Tukey HSD to test for differences between specific groups (Hsu 1996).

The structure of the three ANOVA models follows. The first is a site-by-site comparison across all temperature treatments, using the clade mean at each site. This gives an effective sample size of five replicates. The second is a clade-by-clade comparison, in which a separate analysis was performed for each clade, with site and temperature as orthogonal factors. The units of comparison are replicates within each clade at each site, usually five, although there were some lost samples (see below.) The third consists of three experiment-wide ANOVAS at 4, −5, and −10°C. The unit of comparison is replicate within clade.

All statistical analysis was conducted in the R statistical environment (R Development Core Team 2011). Because the response to temperature varied between quasi-linear (ex, Fabaceae and Pittosporaceae at the temperate site, Figure 13) and strongly sigmoidal (ex, Myrtaceae at both wet

Figure 13: Temperature response by site and clade Top: Each line shows the mean response to nadir temperature. Bottom: Each line shows the average decrease from the initial measurement at each nadir temperature. For sample sizes, see *Statistical analysis,* Methods section.



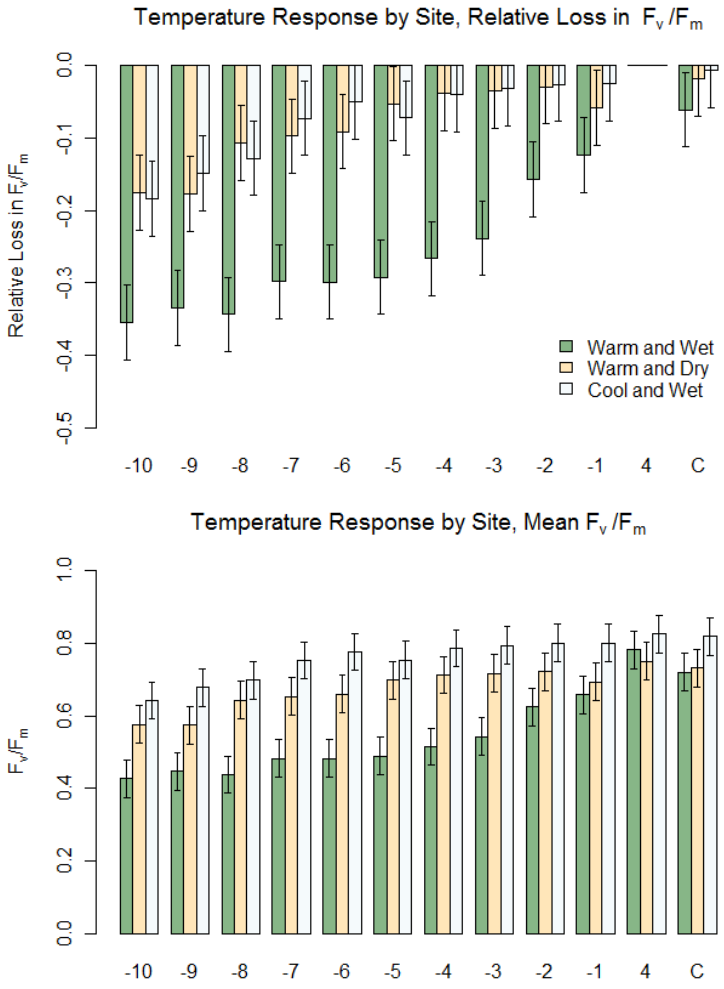
sites) temperature was treated as a factor rather than as a continuous variable. Sample sizes are as follows in all figures and tables: $n = 1$ for Rhamnaceae or Oleaceae from the wet tropical site; these were never included in the statistical analyses. There is no data point for Rhamnaceae at −8°C the wet tropical site, due to loss of the entire sample rack; no error bars are shown in figures and no comparisons were made to other points. All other groups are $n = 5$ except Rubiaceae at −1° and −10°C from the dry tropical site, and the Rubiaceae positive control from the temperate site, which each have $n = 4$.

Results

Site-by-site comparisons

There were clear differences between both sites and clades (Figure 13). As hypothesized, freezing was the most deleterious to samples from the wet tropical sites. However, a surprising pattern appeared at the temperate site. All taxa from this site were not consistently resistant to freezing. The coldest temperature reported near the temperate site is −6.7°C. Above this temperature, samples from this site showed very little damage. However, below this temperature, some taxa showed large amounts of damage, while others showed very little.

Figure 14: Temperature response by site Mean F_v/F_m, top, and relative loss in F_v/F_m, bottom. Error bars show standard error of the mean, as the Site × Temperature interaction was not significant. For sample sizes, see *Statistical analysis*, Methods section.



ANOVA indicated highly significant effects of both temperature and site, but no significant interaction, in both the mean value of, and relative loss in, F_v/F_m (Figure 14; Table 16). Tukey HSD indicated the wet tropical site was significantly different from the cold site and the dry site, but that the cold site and the dry sites were not significantly different from one another. Tests on both mean F_v/F_m and relative loss in F_v/F_m demonstrated that the response of samples from the dry tropical site was much more similar to the temperate site than to the wet tropical site. Indeed, relative loss in F_v/F_m was statistically indistinguishable between temperate

Table 16: Differences between sites ANOVA results and Tukey HSD comparisons, for five species means at three sites and twelve temperatures. Top: mean F_v/F_m. Bottom: relative loss in F_v/F_m.

ANOVA, mean F_v/F_m

	df	*Sum Sq*	*Mean Sq*	*F*	*p*	
Site	2	1.343	0.671	28.473	$3.81e^{-11}$	***
Temp	11	0.894	0.081	3.449	$2.78e^{-4}$	***
Site × Temp	22	0.241	0.011	0.465	0.98	
Residuals	144	3.395	0.024			

Tukey HSD, mean F_v/F_m

	difference	*lower bound*	*upper bound*	*adj. p*	
WD-WW	0.127	0.061	0.193	$3.62e^{-5}$	***
CW-WW	0.21	0.144	0.276	$< 2e^{-16}$	***
CW-WD	0.083	0.017	0.149	0.01	*

ANOVA, relative loss in F_v/F_m

	df	*Sum Sq*	*Mean Sq*	*F*	*p*	
Site	2	1.046	0.523	39.351	$2.32e^{-14}$	***
Temp	11	0.894	0.081	6.117	$3.52e^{-8}$	***
Site × Temp	22	0.241	0.011	0.825	0.691	
Residuals	144	1.914	0.013			

Tukey HSD, relative loss in F_v/F_m

	difference	*lower bound*	*upper bound*	*adj. p*	
WD-WW	0.158	0.108	0.208	$< 2e^{-16}$	***
CW-WW	0.165	0.116	0.215	$< 2e^{-16}$	***
CW-WD	0.008	–0.042	0.058	0.927	

and dry tropical sites (Table 16). Comparisons of mean F_v/F_m showed no significant difference between the two control treatments at any site ($p > 0.99$ for all fifteen pairwise comparisons, Tukey HSD.)

Within-clade comparisons

Within clades, taxa from different seasonality environments varied in their responses. Figure 15 shows significant differences in the site-by-temperature interaction for each clade as least significant difference (LSD) error bars, and Table 17 summarizes the results of ANOVA and Tukey HSD comparisons. Site and temperature were significant in all comparisons. In most cases, the two seasonal sites were more similar to one another than either was to the wet tropical site, as in the site-by-site comparison above. This was observed either as significant differences between all three sites, with the smallest magnitude between the two seasonal sites, or as a significant difference only between the wet tropical site and the seasonal sites. The exception to this pattern was Pittosporaceae; in this relatively resistant clade, samples from the dry tropical site were generally more similar to the wet tropical site. Significant interactions (Table 17) were associated with two patterns: a very shallow slope in response to temperature at the temperate site (Fabaceae and Pittosporaceae) or a very steep slope at the wet tropical site (Rubiaceae and Myrtaceae.) Of the clades with full sampling, only Rutaceae, with no statistical difference between sites at most temperatures, lacked a significant interaction.

Together, clades show two distinct patterns, which reflect the statistically significant differences between groups (see *Comparison between clades*, below.) For one set of clades, there was little or no statistical difference between samples from all three sites at each temperature, and the total relative loss in F_v/F_m was small. I call these 'resistant clades.' This group includes Fabaceae and Pittosporaceae; Rutaceae was not as resistant, but was more resistant than the remaining clades. Fabaceae and Pittosporaceae collections from all three sites never fell below an average F_v/F_m of 0.598 and 0.600 respectively, at any temperature.

The other set of clades showed a large overall decline: these are 'vulnerable clades.' At most temperatures, clades in this group show little or no significant difference between the temperate site and the dry tropical site, but a large and highly significant difference between the wet tropical site and the other two sites. The vulnerable clades are typified by Rubiaceae and Myrtaceae. Oleaceae

Table 17: Anova, relative loss in F_v/F_m for each clade For Rhamnaceae and Oleaceae, statistical analysis was carried out only on data from the dry tropical and temperate sites.

Fabaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	2	0.084	0.042	16.53	$3.92e^{-7}$ ***	WD-WW	0.038	0.015	0.060	$4.36e^{-4}$ ***
Temp	10	0.357	0.036	14.06	$< 2.2e^{-16}$ ***	CW-WW	0.054	0.031	0.077	$3.44e^{-7}$ ***
Site × Temp	20	0.167	0.008	3.29	$2.14e^{-5}$ ***	CW-WD	0.016	–0.006	0.039	0.208
Residuals	132	0.335	0.003							

Pittosporaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	2	0.155	0.077	27.95	$7.58e^{-11}$ ***	WD-WW	0.034	0.010	0.058	0.002 **
Temp	10	0.301	0.030	10.88	$2.43e^{-13}$ ***	CW-WW	0.075	0.051	0.099	$2.98e^{-11}$ ***
Site × Temp	20	0.119	0.006	2.15	0.005 **	CW-WD	0.041	0.017	0.064	$2.52e^{-4}$ ***
Residuals	132	0.365	0.003							

Rhamnaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	1	0.016	0.016	5.84	0.018 *	CW-WD	–0.025	–0.046	–0.004	0.018 *
Temp	9	1.241	0.138	49.72	$< 2.2e^{-16}$ ***					
Site × Temp	9	0.071	0.008	2.84	0.006 **					
Residuals	80	0.222	0.003							

Rutaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	2	0.263	0.131	18.61	$7.56e^{-8}$ ***	WD-WW	0.067	0.029	0.105	$1.60e^{-4}$ ***
Temp	10	0.940	0.094	13.31	$6.39e^{-16}$ ***	CW-WW	0.095	0.057	0.133	$7.08e^{-8}$ ***
Site × Temp	20	0.197	0.010	1.40	0.134	CW-WD	0.028	–0.010	0.066	0.184
Residuals	132	0.932	0.007							

Oleaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	1	0.212	0.212	24.69	$3.29e^{-6}$ ***	CW-WD	0.088	0.053	0.123	$3.28e^{-6}$ ***
Temp	10	0.435	0.043	5.07	$8.33e^{-6}$ ***					
Site × Temp	10	0.102	0.010	1.19	0.306					
Residuals	88	0.756	0.009							

Rubiaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	2	4.836	2.418	164.90	$< 2.2e^{-16}$ ***	WD-WW	0.345	0.290	0.400	$3.55e^{-15}$ ***
Temp	10	0.686	0.069	4.68	$1.07e^{-5}$ ***	CW-WW	0.380	0.326	0.435	$3.55e^{-15}$ ***
Site × Temp	20	0.711	0.036	2.42	0.002 **	CW-WD	0.036	–0.020	0.091	0.281
Residuals	130	1.906	0.015							

Myrtaceae

	ANOVA						Tukey HSD			
	df	*Sum Sq*	*Mean Sq*	*F*	*p*		*difference*	*lower bound*	*upper bound*	*adj. p*
Site	2	3.871	1.935	135.51	$< 2.2e^{-16}$ ***	WD-WW	0.359	0.305	0.413	$< 1.0e^{-7}$ ***
Temp	10	2.438	0.244	17.07	$< 2.2e^{-16}$ ***	CW-WW	0.273	0.219	0.327	$< 1.0e^{-7}$ ***
Site × Temp	20	1.230	0.061	4.30	$1.48e^{-7}$ ***	CW-WD	–0.086	–0.140	–0.032	$7.35e^{-4}$ ***
Residuals	132	1.885	0.014							

also appears to follow this pattern, although low sample size at the wet tropical site limits the conclusions that can be drawn about this clade. It should be noted, however, that Oleaceae suffered more damage than any other clade at the dry tropical site, where it had full replication. This is a potentially interesting countervailing pattern, and the only exception in this data set to a near-match in tolerance between temperate, and dry tropical, collections. With the same caveat about sample

size, Rhamnaceae appears to be intermediate between the two groups: collections from all three sites were resistant to ~−6°C; below this temperature, samples from the cold site plunge to damage levels below even those observed at the wet tropical site

Comparison between clades

To test whether clades responded differently to temperature treatments, clade and site were compared simultaneously at three different points in the experiment: the positive control,

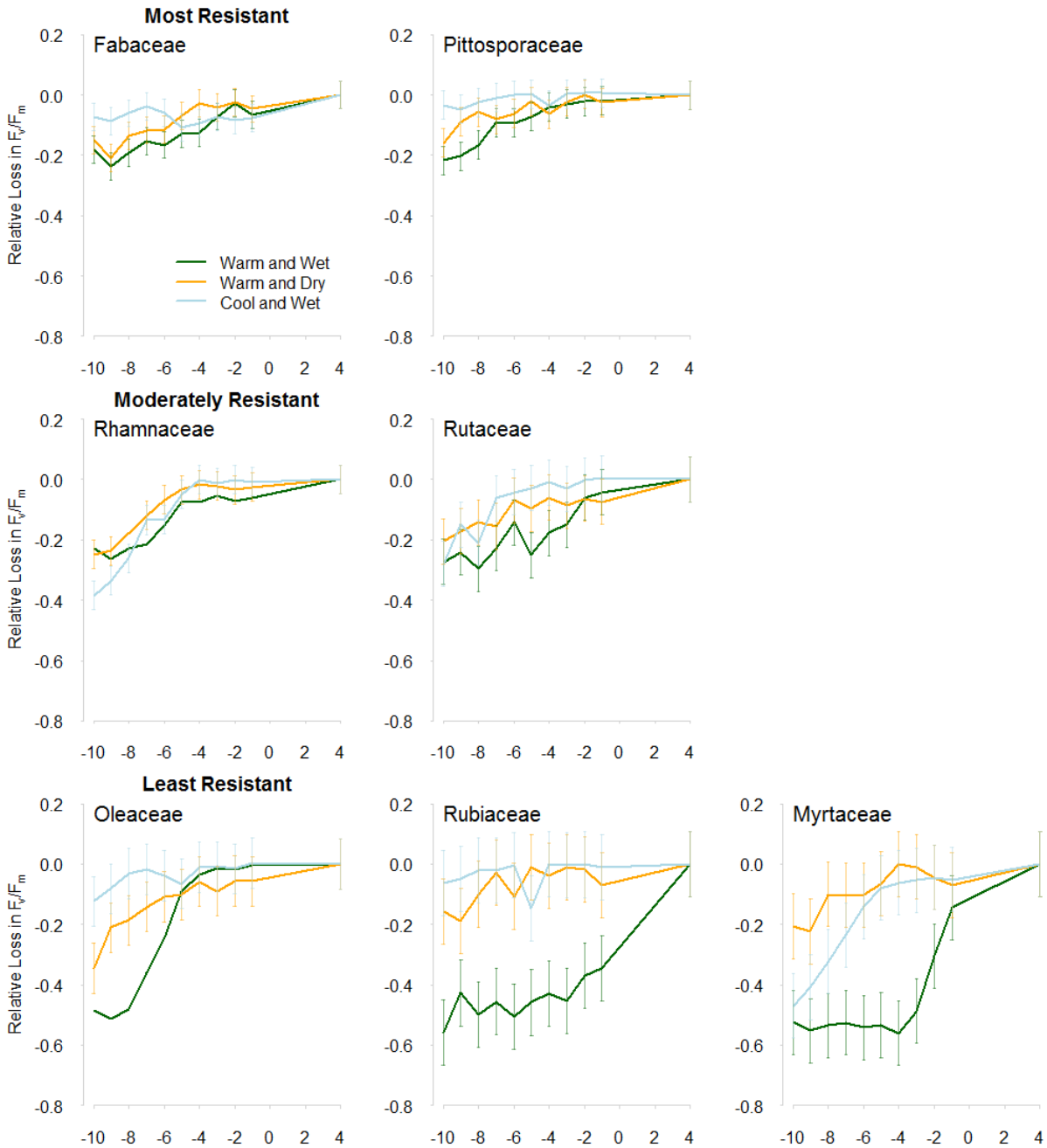


Figure 15: Response by clade at each site, relative loss in F_v/F_m Error bars show least significant difference between Site × Temperature interaction levels. Only the 4°C control is shown; the positive control is not shown. For sample sizes, see *Statistical analysis*, Methods section.

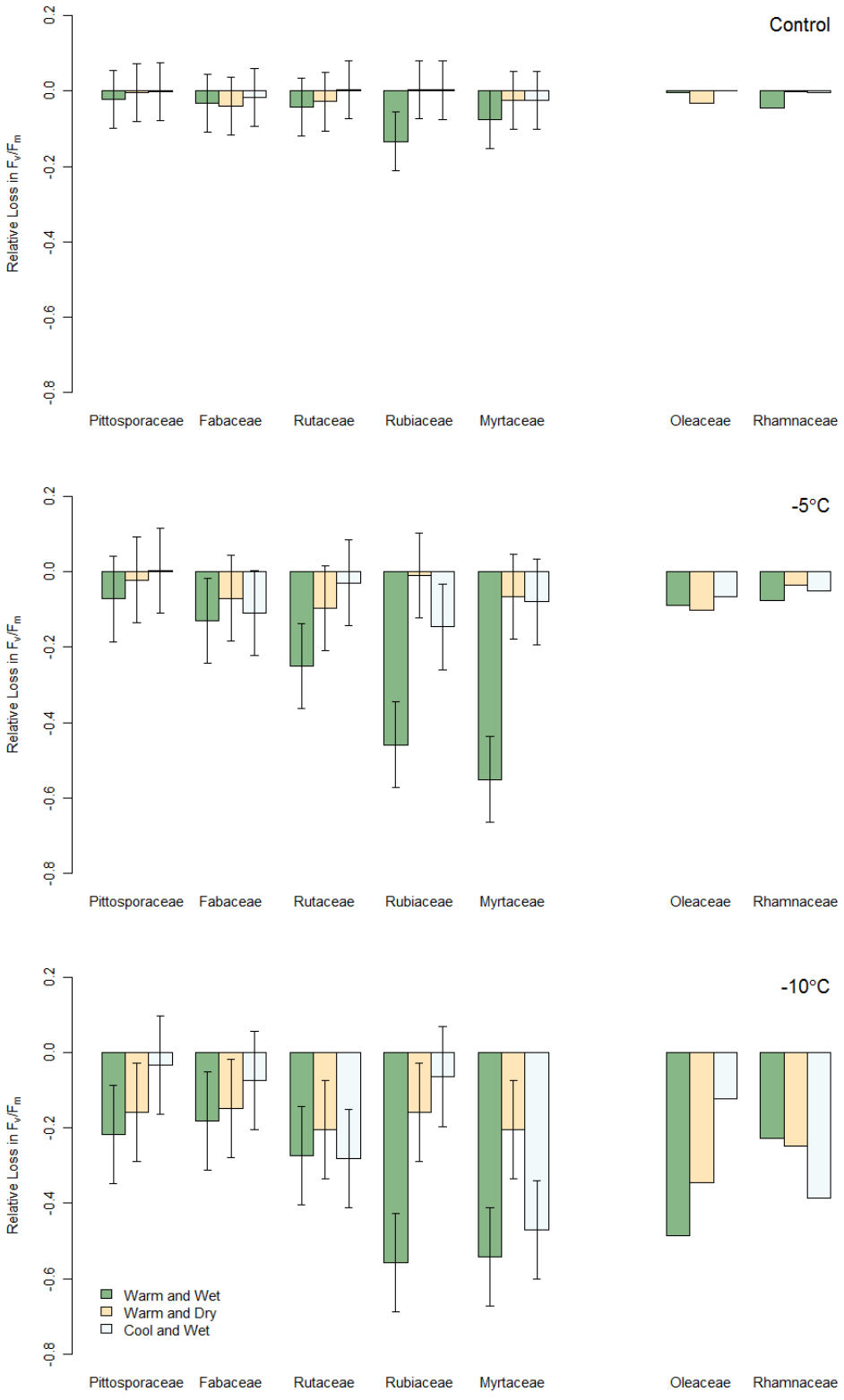


Figure 16: Interactions of clade and site at three different temperatures Error bars show least significant difference between interaction levels; non-overlapping bars indicate $p < 0.05$. For sample sizes, see *Statistical analysis*, Methods section**.**

Table 18: ANOVA, clade by site interaction under control, −5°, and −10°C treatments

	Control				
	df	*Sum Sq*	*Mean Sq*	*F*	*p*
Site	2	0.039	0.020	4.61	0.014 *
Clade	4	0.012	0.003	0.72	0.583
Site × Clade	8	0.039	0.005	1.13	0.354
Residuals	59	0.252	0.004		
	−5				
	df	*Sum Sq*	*Mean Sq*	*F*	*p*
Site	2	0.887	0.443	47.66	$4.05e^{-13}$ ***
Clade	4	0.396	0.099	10.65	$1.38e^{-6}$ ***
Site × Clade	8	0.560	0.070	7.53	$6.64e^{-7}$ ***
Residuals	60	0.558	0.009		
	−10				
	df	*Sum Sq*	*Mean Sq*	*F*	*p*
Site	2	0.506	0.253	20.54	$1.70e^{-7}$ ***
Clade	4	0.752	0.188	15.25	$1.28e^{-8}$ ***
Site × Clade	8	0.630	0.079	6.39	$5.91e^{-6}$ ***
Residuals	59	0.727	0.012		

Table 19: Tukey HSD comparison of differences between sites under control, −5°, and −10°C treatments.

	Control			
	difference	*lower bound*	*upper bound*	*adj. p*
WD-WW	0.042	–0.002	0.087	0.066
CW-WW	0.054	0.009	0.098	0.015 *
CW-WD	0.011	–0.034	0.056	0.815
	−5			
	difference	*lower bound*	*upper bound*	*adj. p*
WD-WW	0.240	0.174	0.305	$2.66e^{-11}$ ***
CW-WW	0.220	0.155	0.286	$1.29e^{-10}$ ***
CW-WD	–0.019	–0.085	0.046	0.756
	−10			
	difference	*lower bound*	*upper bound*	*adj. p*
WD-WW	0.179	0.103	0.255	$1.53e^{-6}$ ***
CW-WW	0.171	0.095	0.246	$3.19e^{-6}$ ***
CW-WD	–0.008	–0.084	0.068	0.966

undamaged leaves; −5°C, close to the coldest occurring temperature at the cold site; and −10°C, ~90% loss of free water in living cells (Thomashow 1990). Table 18 gives ANOVA results for each treatment; Tables 19 and 20 report Tukey HSD results for differences between sites and families respectively. Differences between interaction levels are shown as LSD error bars in Figure 16.

The control measurement showed no significant differences between clades, and no significant clade-by-site interaction. There was a significant effect of site ($p = 0.014$, Table 18) due to low values at the wet tropical site (Table 19). At both of the other temperatures, differences were observed between the clades, with some showing more tolerance to cold than others, even when harvested from a site where they could not have acclimated to freezing temperatures (Figure 16).

At −5°C, there are significant effects for site, clade and the clade-by-site interaction (Table 18), indicating that relative loss of F_v/F_m depends on phylogenetic relationship and seasonality simultaneously. Differences in cold tolerance among the clades (Table 20) correspond to the qualitative differences between resistant and vulnerable groups outlined above. Fabaceae and Pittosporaceae clearly fall into the resistant category: collections from all three sites are not statistically different (Figure 16), and the clade means are not different ($p = 0.239$, Table 20). Species from Rubiaceae and Myrtaceae belong to the vulnerable category; they are not statistically different from one another ($p = 0.937$) but are highly significantly different from both Fabaceae and Pittosporaceae (Table 20). Rutaceae is caught in the middle—significantly different only from Myrtaceae. As in the overall analysis, the significant site effect is due to low values at the wet tropical site—there is no statistical difference between the two seasonal sites (Table 19).

At −10°C, the temperature at which most osmotically active water is withdrawn from the cell, damage to all clades is greater and the boundaries between 'resistant' and 'vulnerable' become blurred. The main effects of clade and site, and the clade-by-site interaction, remain highly significant (Table 18). Increasing damage in the dry tropical, and temperate, collections, however, gives each clade a unique pattern, resulting in only two non-significant Tukey HSD comparisons: a resistant Fabaceae and Pittosporaceae, and a relatively intermediate Rutaceae and Rubiaceae (Figure

16; Table 20). Damage to Myrtaceae is now so severe that it is significantly different from all other clades, most likely due to the fact that both wet tropical, and temperate, collections show a large loss in F_v/F_m.

Table 20: Tukey HSD comparison of clade responses to control, −5°, and −10°C treatments

	Control			
	difference	*lower bound*	*upper bound*	*adj. p*
Myrtaceae-Fabaceae	–0.012	–0.079	0.055	0.985
Pittosporaceae-Fabaceae	0.021	–0.046	0.088	0.907
Rubiaceae-Fabaceae	–0.014	–0.083	0.054	0.976
Rutaceae-Fabaceae	0.007	–0.060	0.074	0.999
Pittosporaceae-Myrtaceae	0.033	–0.034	0.100	0.639
Rubiaceae-Myrtaceae	–0.002	–0.070	0.066	1.000
Rutaceae-Myrtaceae	0.019	–0.048	0.086	0.930
Rubiaceae-Pittosporaceae	–0.035	–0.103	0.033	0.604
Rutaceae-Pittosporaceae	–0.014	–0.081	0.053	0.977
Rutaceae-Rubiaceae	0.021	–0.047	0.089	0.908
	−5			
	difference	*lower bound*	*upper bound*	*adj. p*
Myrtaceae-Fabaceae	–0.129	–0.228	–0.030	0.005 **
Pittosporaceae-Fabaceae	0.073	–0.026	0.173	0.239
Rubiaceae-Fabaceae	–0.102	–0.201	–0.003	0.042 *
Rutaceae-Fabaceae	–0.022	–0.121	0.077	0.971
Pittosporaceae-Myrtaceae	0.202	0.103	0.301	< 1e–7 ***
Rubiaceae-Myrtaceae	0.027	–0.072	0.126	0.937
Rutaceae-Myrtaceae	0.107	0.008	0.206	0.028 *
Rubiaceae-Pittosporaceae	–0.175	–0.274	–0.076	< 1e–7 ***
Rutaceae-Pittosporaceae	–0.095	–0.194	0.004	0.065
Rutaceae-Rubiaceae	0.080	–0.019	0.179	0.170
	−10			
	difference	*lower bound*	*upper bound*	*adj. p*
Myrtaceae-Fabaceae	–0.272	–0.386	–0.158	< 1e–7 ***
Pittosporaceae-Fabaceae	–0.002	–0.116	0.112	1.000
Rubiaceae-Fabaceae	–0.128	–0.244	–0.012	0.023 *
Rutaceae-Fabaceae	–0.118	–0.232	–0.004	0.039 *
Pittosporaceae-Myrtaceae	0.270	0.156	0.384	< 1e–7 ***
Rubiaceae-Myrtaceae	0.143	0.027	0.260	0.008 **
Rutaceae-Myrtaceae	0.154	0.040	0.268	0.003 **
Rubiaceae-Pittosporaceae	–0.126	–0.242	–0.010	0.026 *
Rutaceae-Pittosporaceae	–0.116	–0.230	–0.002	0.044 *
Rutaceae-Rubiaceae	0.010	–0.106	0.126	0.999

Discussion

This study set out to test two hypotheses: 1) Plants from the dry tropical site will show greater tolerance to freezing than plants collected from the wet tropical site, in spite of the fact that they have not been acclimated to freezing temperatures; 2) There will be phylogenetic trends in tolerance to cold—some clades will be more resistant to cold than others, independent of the conditions from which they were collected. Both of these initial hypotheses were upheld.

Seasonal drought can confer resistance to freezing in plants from a tropical environment

Plants from the wet tropical site suffered the most damage when subjected to freezing, and plants from the cold site were very resistant to cold temperatures within the range experienced in their home climate. Plants from the dry tropical site were significantly more resistant to cold than their relatives at the wet tropical site, and they were resistant to temperatures far lower than those found in their local climate. In fact, in several clades, plants from the dry environment had similar or even greater tolerance to temperatures below −6°C than their relatives harvested from the cold environment—a result that was not anticipated. This is even more surprising in light of the fact that collections from the temperate site were exposed to freezing temperatures many times in the month before collection (Australian Government Bureau of Meteorology 2011).

Given the established links between the physiology of drought and freezing (Close 1997; Thomashow 1999; Hara 2010), and the rainfall and temperature regimes near both sites in the month leading up to the experiment (Table 14), the resistance shown by the dry tropical collections can most likely be attributed to drought acclimation at the dry tropical site. The best-demonstrated association between drought and freezing is the induction of dehydrin genes by water deficit, and

evidence that these molecules function in cryoprotection (Close 1997; Thomashow 1999). Although these responses have been studied primarily in model plant systems, there is evidence that they are widespread within plants as a whole (Dure *et al.* 1989; Hoekstra, Golovina, & Buitink 2001). As exposure to chilling clearly does not induce acclimation to freezing in all plants, it is possible that in cases where it does, it represents sub-functionalization within this large gene family (Force *et al.* 1999; Bies-Etheve *et al.* 2008). In support of this, there is evidence that the association of dehydrins with desiccation tolerance may be much older, as this response is present in early-diverging land plants such as mosses (Cuming 2009).

Close relatives share patterns of cold resistance

Clear differences were recorded among members of the different clades studied, with some showing strong tolerance to freezing regardless of collection environment, and others showing very weak tolerance to freezing, even when collected from a seasonally cold environment. Within these categories, two interesting patterns emerged. In clades that appear to be relatively resistant to freezing, there was little or no significant difference between samples from each site at any given nadir temperature, indicating that neither of the two warm sites suffered more damage than the cold site. In the case of Pittosporaceae, samples from the dry tropical site were most similar to samples from the wet tropical site. Apparently, in this clade, which shows a high resistance to frost, drought did not impart any additional acclimation to freezing temperatures. The opposite pattern was observed for collections from the clades that appear to resist freezing poorly, in which the response of samples from the dry tropical site was similar to that of samples from the temperate site. This suggests that it is in these more vulnerable clades that drought has the most potential protective effect against subsequent freezing damage.

The relative differences between the clades were noticeable in another aspect of the experiment. Species from the cold site were generally able to resist freezing well, as would be expected given the climatic regimes at the sites where they were collected. Indeed, given that the coldest temperature recorded near the cold site in the past 40 years is only −6.7°C, most are over-engineered with respect to the level of cold experienced in their home environment. Members of Rhamnaceae and Myrtaceae, however, appear to have a very small margin of safety. Cold-climate collections from both of these clades suffered dramatically at temperatures below −6°C, underperforming collections from the warm-and-dry site. In fact, temperate Rhamnaceae collections actually suffered more freezing damage, in absolute terms, than the single collection from the wet tropical site! The tendency for species in these clades to be vulnerable to freezing appears to affect even members of these groups that have adapted to cold environments. This suggests that the underlying metabolic, structural, or physiological traits that cause the species in these clades to be vulnerable are difficult to change, either through plasticity or adaptation.

The vulnerability of Myrtaceae is particularly interesting. Myrtaceae is one of the world's most speciose plant families, (#9 in the top 10, APG III) but most of its diversity is tropical or subtropical (Sytsma *et al.* 2004). In working with both Myrtaceae and Rutaceae, two of the most vulnerable clades in the experiment, one of the most noticeable aspects of leaf structure in both was the

presence of large oil glands. Perhaps some aspect of producing these secondary compounds—either an effect on leaf structure, or on the sources of materials available to produce other compounds related to freeze-tolerance—makes it difficult for plants with this characteristic to adapt to low temperatures. Conversely, however, the low-temperature biology of *Eucalyptus pauciflora*, a member of Myrtaceae, is well-studied (Ball, Hodges, & Laughlin 1991; Ball *et al.* 1997, 2002a, 2004; Blennow *et al.* 1998; Roden, Egerton, & Ball 1999; Atkin, Holly, & Ball 2000). Known as 'snow gum' in Australia, acclimated leaf tissue from this species has been reported to withstand temperatures as low −23°C (Sakai, Paton, & Wardle 1981).

Another notable result from the clade-by-clade comparison is that the two most resistant clades, Fabaceae and Pittosporaceae, were also the two clades sampled at the generic level—*Acacia* and *Pittosporum*, respectively. Taxa from these clades were the closest relatives compared in this study. This may suggest that these genera have (or have had) an exaptation for surviving low temperatures, which allowed them to rapidly colonize many different environments. Both clades are highly diverse within Australia, and, unlike many of the other genera considered in this study, are found in dry and arid environments well outside the range of the rainforest habitats considered here. Although both are originally thought to be descended from Gondwanan rainforest ancestors (Truswell 1993; Chandler *et al.* 2007), their most recent common ancestor may have been a cold- or drought-adapted species which dispersed back into the tropical rainforest. Interestingly, at least one biogeographical study has suggested a *Eucalyptus-Acacia* ecotone in Australian woodlands based on low temperature (Bowman & Connors 1996).

Conclusions

Could a seasonally dry environment provide an adaptive stepping stone between wet tropical rainforest and a seasonally-cold temperate environment? The evidence presented here demonstrates that living tissue of drought-acclimated flowering plants from a variety of phylogenetic backgrounds shows a surprising tolerance of freezing temperatures. It also demonstrates that this is true even in the absence of conditions allowing for frost acclimation. Therefore, this study demonstrates that this is a potentially plausible pathway between the tropics and the temperate.

A number of other factors must be considered, however. The potential for multiple dispersals between tropical everwet and seasonal environments is particularly important. It is clear that previous relevant adaptive environments can have an important influence on where taxa establish and thrive (Harvey & Pagel 1991; Ricklefs & Latham 1992; Peterson, Soberón, & Sánchez-Cordero 1999; Ackerly 2003, 2004). Although global climate history and phylogeny make a strong case for the directionality of angiosperm evolution from tropical and aseasonal to temperate and seasonal (Mathews & Donoghue 1999; Qiu *et al.* 1999, 2005; Crowley 2001; Zachos *et al.* 2001), this cannot uncritically be assumed to have been the path that taxa in this study have taken. Instead, this approach should be considered a modern analog, testing whether a proposed historical mechanism is possible or not.

This study raises a number of interesting directions for future research. A number of taxa—even those from the wet tropical site—showed a surprising level of resistance to frost given their home

environments. This has previously been observed in other Southern Hemisphere taxa (Bannister & Lord 2006; Bannister 2007). This pattern merits further exploration, especially through common-garden approaches incorporating *in vivo* cold-acclimation. It was also interesting to find cold-climate taxa living near the edge of their ability to tolerate frost. Research in this direction might explore the reasons for such a fine balance, from both the perspective of functional cost/benefit trade-offs and minima in the adaptive landscape. It might also ask whether these taxa are new, in evolutionary terms, to the environments they inhabit. It would be worthwhile to find out whether this is a stable balance, or directional selection in action.

The physiological evidence presented here from modern-day environments suggests an interesting pattern to be explored further at larger scales. To answer this question definitively will require exploring many more species and individuals, using detailed evidence from both fossil and phylogenetic sources. In particular, the constantly-improving field of phylogenetic dating will be of great importance to studying these hypotheses in the future. Specifically, the ability to trace the relevant adaptive environment of multiple common ancestors will greatly illuminate our ability to follow the evolutionary path of individual lineages through time and across climates.

Conclusion

The ancestral angiosperm niche was most likely tropical (Hickey & Doyle 1977; Gübeli, Hochuli, & Wildi 1984; Crane & Lidgard 1989; Wiens & Donoghue 2004; Feild *et al.* 2004). From these origins, angiosperms dispersed around the world under the warm conditions that prevailed during the Late Cretaceous, Paleocene, and Eocene (Crane & Lidgard 1989; Wing & Boucher 1998; Barrett & Willis 2001; Willis & McElwain 2002). As the climate became colder during the later Tertiary and Quaternary, these widespread lineages faced new conditions. The work presented here addresses the profound impact of this particular historical path on this large and important clade. Chapter 1 demonstrated that latitudinal span is strongly associated with diversification, and that, in an evolutionary context, this is stronger than any association with range area. In Chapter 2, I explored the roots of this diversification, by examining how functional diversity might increase in response to seasonality, and by investigating how local adaptation and niche conservatism interact across these gradients. Chapter 3 tested a specific hypothesis behind tropical to temperate transitions, and showed that seasonally dry conditions can act as a physiological stepping stone to surviving freezing.

Comments on Chapter 1: Modes of diversification related to latitude

In Chapter 1, I investigated a large-scale question: to what extent have area, seasonality, and time influenced the patterns of species diversity that characterize the present-day world? My results show a strong association between rates of diversification and the crossing of latitudinal boundaries. This suggests that the number environments and selective pressures to which the clade is exposed can affect how many speciation events take place. This interpretation is further supported by a small, but significant effect showing increased diversification in response to cooler temperatures and greater seasonality.

What might have caused these patterns? It is tempting to think the tropical origin of angiosperms means they dispersed across steep latitudinal temperature gradients such as those observed today. However, climate history refutes this view: by contrast, it is now clear that the world was warm and equable during the first ~170 Ma of angiosperm evolution. Angiosperms clearly had a global range well before the Eocene-Oligocene climatic transition (Crane & Lidgard 1989; Wing & Boucher 1998; Barrett & Willis 2001; Willis & McElwain 2002). Instead of dispersing into seasonal climates, ancestral flowering plants were probably exposed to changing climates *in situ*. These results, which show that speciation is correlated with latitudinal span, may be evidence to support a 'trailing edge' hypothesis (Ackerly 2003). Under this scenario, populations at the novel end of a shifting climate gradient are the most likely to undergo adaptive change. From the Oligocene to the industrial era, this would have been the cooler end of the gradient in temperature: higher latitudes. This is in accord with results from Chapter 1.

It is worth asking which specific functional shifts were involved in these patterns. Adaptations associated with tolerance or avoidance of the unfavorable season would have been favored. Mechanisms of tolerance are complex, and Chapter 3 discusses one pathway by which they may have appeared. There may also have been selection for avoidance, in the form of shorter lifespan

and faster reproduction. This results in an annual lifestyle, which allows plants to overwinter as seeds. Two external pieces of evidence support this. First, there is a sharp break in the latitudinal gradient in both seed size and plant height between 20° and 25° latitude, which is strongly associated with turnover from floras numerically dominated by woody plants, to floras numerically dominated by herbaceous plants (Moles *et al.* 2007, 2009). It is possible that the higher relative number of herbaceous plants observed in temperate floras is the result of selection for overwintering as a seed. Indeed, many large clades of flowering plants consist of mostly herbaceous clades sister to, or nested within, woody tropical groups (Judd, Sanders, & Donoghue 1994; Smith & Donoghue 2008). Second, there is a strong link drawn between the shorter generation times associated with herbaceous plants and higher rates of both molecular evolution and diversification (Tuskan *et al.* 2006; Smith & Donoghue 2008). This suggests that selection for herbaceous, annual life history strategies is, in itself, selection for higher diversification rates.

If this is true, it may have profoundly shaped patterns of functional and evolutionary diversity in angiosperms today. Indeed, it is possible that herbaceous forms contribute to the anomalously high species diversity of angiosperms as a group (Davies *et al.* 2004a; Smith & Donoghue 2008; Crepet & Niklas 2009). Several authors have found that clades containing herbaceous members tend to be more diverse than woody clades (Ricklefs & Renner 1994, 2000; Dodd, Silvertown, & Chase 1999). Diversity of life form is markedly different between the highly diverse angiosperms and the relatively depauperate gymnosperms. Gymnosperms have trees: angiosperms have trees, vines, geophytes, lianas, epiphytes, parasites, and annuals (Rowe & Paul-Victor 2012). In keeping with this, angiosperms have a remarkable ability to loose and gain (or regain) wood in response to environmental conditions (Carlquist 1969; Bate-Smith 1972; Kalkman 1988; Wojciechowski *et al.* 2000; Takamatsu, Hirata, & Sato 2000; Lens, Smets, & Melzer 2012). Ferns have more functional diversity than gymnosperms—in addition to rhizomatous and rosette plants, there are arborescent forms, aquatic herbs and at least one twining form. Even 'tree' ferns, however, lack well-developed secondary growth, and the competitive advantages it can confer through increased height (Westoby 1998; Falster & Westoby 2003; Westoby & Wright 2006). The ability of angiosperms to grow as both trees and herbs, and to switch between these two forms, appears to have allowed them to exploit many niches not open to other plants. If seasonal conditions helped select for woody to herbaceous transitions in some clades, this may have contributed to the diversity seen in this group as a whole.

Comments on Chapter 2: Niche axis length and trait conservatism

Chapter 2 focused on a specific example of tropical to temperate transitions. It showed that functional diversity within a community can increase in response to seasonality, and that greater species diversity was not associated with higher functional diversity. Secondly, it explored long-standing questions about the relative role of phylogenetic niche conservatism and local adaptation in community assembly at continental scales (Travis 1989; Harvey & Pagel 1991; Lord, Westoby, & Leishman 1995; Webb *et al.* 2002; Ackerly 2003, 2004). A role was found for both, but the relative importance of each process varied greatly with the trait explored. Traits relating to successional

strategy were generally highly conserved, while traits relating to carbon capture and water use were not.

Classical views of niche differentiation suggest that in order to coexist, species must exploit different aspects of their environment (Hutchinson 1957; Hardin 1960). Three different hypotheses potentially explain how some environments can support many more species than others: 1) Niche breadth—the amount by which species must differ in optimal resource use—may be the same, but organisms in the high-diversity environment are able to exploit a greater range of values, leading to a longer community-wide niche axis. This would mean a greater total range of resource use is observed in diverse communities, and this would be reflected in a greater range of trait values. Such a pattern could be due either to greater availability of resources, or to a greater ability to exploit the environment due to higher differentiation (Connell & Orias 1964; MacArthur 1969; Connell 1978; Ricklefs 1987). 2) The community-wide niche axis may not be longer than in the low diversity environment, but niches themselves may be narrower. This suggests that species may be more highly specialized in more diverse environments (Janzen 1967; MacArthur 1984; May 2001; Vázquez & Stevens 2004; Ghalambor *et al.* 2006). 3) Both the community-wide niche axis and the breadth of each individual niche may be similar to that observed in lower diversity environments, but the niches may have greater overlap. This implies that more species co-exist at any given resource value (Klopfer & MacArthur 1961; May & Arthur 1972; Ricklefs 1987).

Results from Chapter 2 join other data in rejecting the first hypothesis (Condit *et al.* 2006; Clark *et al.* 2007). In no case was a greater range of trait values observed in the highly-diverse wet tropical community. At present, these results cannot differentiate between the second and third hypotheses. However, future research expanding on the data presented here could do so. Measuring several sites across resource gradients in each of the three environments would make it possible to quantify the niche breadth of each species considered. This would enable comparisons of the relative overlap between different species, and the specialization of each species.

The second part of Chapter 2 compared the relative contributions of phylogenetic niche conservatism and local adaptation to trait differences at large scales. Phylogenetic niche conservatism is the tendency for species to stay in the same habitat as their ancestors. At community scales, it is a force for stabilizing selection: across a landscape, in the absence of significant dispersal limitation, each microsite will be occupied by a species that is near its optimal environmental niche (Travis 1989; Harvey & Pagel 1991; Webb *et al.* 2002; Ackerly 2003, 2004). At the same time, at scales where dispersion limitation applies, such perfect sorting is not possible. As a result, at large scales, a mix of local adaptation and phylogenetic trait conservatism should be observed.

Data presented in Chapter 2 come from a continental scale. In the traits examined, it appears that some are highly conserved, while others are highly labile. Traits relating to successional status, particularly wood density (Enquist *et al.* 1999; Muller-Landau 2004; King *et al.* 2006; Chave *et al.* 2009), and pith characters, are highly conserved. This suggests that successional status may be under stabilizing suggestion. SLA and leaf size, which relate to carbon capture, and Huber value, which relates to water use were, by contrast, highly labile. This suggests that they are subject to either

developmental plasticity, or undergo local adaptation quickly. It has also been argued that traits with greater plasticity are more likely to undergo adaptation (Simpson 1953). Yet these results also present a paradox, as both wood density and SLA are well-correlated with growth rate. Perhaps these two traits pertain to different aspects of growth rate—one relating to successional status and the other to carbon-captures strategy, two aligned but not necessarily synonymous aspects of life history strategy.

Whether these are general rules, or specific to the environments investigated here, will require further investigation. Drought forms one of the major contrasts between environments examined in this study. Therefore, it is possible that more selection along axes of water use was detected because it has been an important selective pressure in the environment studied. By contrast, all three of the forests studied were closed-canopy forests. This may explain the relative conservatism of traits relating to successional status within them.

Comments on Chapter 3: Seasonal drought confers resistance to seasonal cold

Chapter 3 convincingly demonstrated that drought can confer unexpected freezing resistance across a range of plant clades. Although I did not demonstrate that the clades studied here followed a path from wet tropical to dry tropical to wet temperate, my data convincingly show that this mechanism of protection is physiologically possible. Two further directions are suggested by this research. One is to explore in detail the evolutionary premise of the experiment; the second is to elucidate the physiological mechanisms that led to the observed result.

From an evolutionary perspective, it would be interesting to know whether the lineages examined in this chapter followed the adaptive pathway outlined, from wet tropical to temperate via a warm, but seasonally dry, environment. Each clade studied is represented only by a polytomy in this analysis. A detailed phylogeny for these groups, including both improved resolution and wider sampling of other clade members, would clarify whether there have been repeated transitions between dry and cold environments. This pattern would support the hypothesis that this has been an important pathway to cold tolerance in the Australian flora. Understanding the recent evolutionary history, and relevant adaptive environments, of the clades studied here through their fossil records would also be an important test of this hypothesis.

It would also be interesting to know if there any evidence for this mechanism in other groups, and across latitudinal gradients on other continents. A very large proportion of the tropical to temperate comparisons of chilling and frost tolerance have been done in Australia or New Zealand (e.g., Read & Hope 1989; Cunningham & Read 2002, 2003; Bannister & Lord 2006). The logistics of performing a similar experiment in North America, Europe, or Asia would be much more complex—it would require working with multiple different permitting and customs agencies. Nonetheless, definitive answers to questions about the differences between tropical and temperate ecosystems will not be possible without such efforts.

Exploring the physiological mechanisms behind the effects observed here will also reward further research. A common garden in which cross-latitudinal collections from several clades were grown,

and acclimated to either freezing or drought, under controlled conditions, could resolve the relative role of acclimation and adaptation in the responses observed. In particular, a factorial experiment by temperature and drought, or reciprocal transplants, would be very informative in separating phylogenetically inherited tolerance to cold and the relative contribution of acclimation to seasonal conditions. This approach would also give each lineage the opportunity to acclimate to cold. In this study, I chose to work with unacclimated plants from the two tropical sites, following the logic that this would be a way to observe the most deleterious possible effects of frost. However, even the wet tropical lineages studied may have some potential to acclimate to frost, given the right conditions. Research suggests that the shared genes involved in drought and cold acclimation have a very ancient origin and are found in all seed plants, so there would be some precedent for such a finding (Thomashow 1999; Eriksson & Harryson 2011).

Although the design of Chapter 3—a factorial cross between clade and seasonality—was highly informative for groups that spanned the entire distance, it necessarily omitted clades that were *not* present at one of the sites. Including such frost-sensitive lineages in a future common garden would give a clear picture of the physiological response of plants that are unable to make the transition from tropical to temperate, and would help delimit the conditions that keep many lineages restricted to the tropics.

Conclusions

Thus, the overarching conclusion of this work is that the transition from tropical and aseasonal to temperate and seasonal has been more than simply a stress through the history of angiosperm evolution. Instead, it has been the driving force for a number of changes that shaped the present-day angiosperm flora. It has contributed to the high rate of diversification observed in angiosperms, and has probably also contributed to the high level of functional diversity that characterizes this clade.

Other forces have evidently been important in shaping the modern diversity of angiosperms. Biotic interactions clearly played a role in promoting species richness, in particular, plant-animal interactions relating to pollination and fruit dispersal (Crepet 1984; Herrera 1989; Eriksson, Friis, & Löfgren 2000; Smith 2001; Bolmgren & Eriksson 2005; Kay & Sargent 2009; Johnson 2010). Mutualistic associations are also important, including those with insects (Lengyel *et al.* 2009), fungi (Knoll & James 1987; Simon *et al.* 1993), and microbes (McKey 1994; Wang *et al.* 2009). However, in concert with these forces, angiosperms have been strongly shaped by climate history. Both phylogenetic niche conservatism in this ancestrally tropical clade (Wiens & Donoghue 2004) and local adaptation under trailing edge conditions are reflected in the species richness, distribution, and functional diversity of this group.

It has frequently been hypothesized that angiosperms are limited by seasonal conditions, and that low species richness at higher latitudes is due to selective pressure is for stress tolerance (Wallace 1878; Dobzhansky 1950; Fischer 1960). By contrast, my work shows that seasonality leads to higher rates of speciation, can contribute to greater functional diversity, and may prepare plants for unexpected challenges. Rather than limiting form and function, it has spurred innovation through the history of flowering plants. This ought to make us reevaluate our definition of what a limiting

condition is: nothing is limiting, for organisms that are adapted to it. Given differential fitness and sufficient time, there is a fine line between *limitation* and *selective pressure*.

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Appendix A

Clades used for the phylogenetic niche conservatism analysis, Chapter 2

Taxa are shown at left, followed by family. Site abbreviations are as follows: CC: Canopy Crane Research Station (wet tropical); FM: Forty Mile NP, (dry tropical); MF: Mt. Field NP, (wet temperate.) Growth habits are shown at right.

1. Bignoniaceae to Lamiaceae			
Callicarpa longifolia	Verbenaceae	CC	Shrub
Glossocarya hemiderma	Verbenaceae	CC	Vine
Neosepicaea jucunda	Bignoniaceae	CC	Vine
Pandorea pandorana	Bignoniaceae	CC	Vine
Pseuderanthemum variabile	Acanthaceae	CC	Herb
Hypoestes floribunda	Acanthaceae	FM	Herb to subshrub
Prostanthera lasianthos	Lamiaceae	MF	Tree
2. *Notelaea*			
Notelaea sp. Cape York	Oleaceae	CC	Tree
Notelaea microcarpa	Oleaceae	FM	Tree
Notelaea ligustrina	Oleaceae	MF	Tree
3. Rubiaceae			
Amaracarpus nematopodus	Rubiaceae	CC	Shrub
Antirhea tenuiflora	Rubiaceae	CC	Shrub
Atractocarpus hirtus	Rubiaceae	CC	Shrub
Hedyotis auricularia	Rubiaceae	CC	Herb
Ixora biflora	Rubiaceae	CC	Shrub
Lasianthus strigosus	Rubiaceae	CC	Shrub
Psychotria dallachiana	Rubiaceae	CC	Shrub
Everistia vacciniifolium	Rubiaceae	FM	Shrub
Psychotria daphnoides	Rubiaceae	FM	Shrub
Psydrax odorata	Rubiaceae	FM	Tree
Coprosma quadrifida	Rubiaceae	MF	Shrub
4. *Pittosporum*			
Pittosporum rubiginosum	Pittosporaceae	CC	Shrub
Pittosporum spinescens	Pittosporaceae	FM	Shrub to tree
Pittosporum bicolor	Pittosporaceae	MF	Tree
5. Ericales			
Ardisia brevipedata	Primulaceae	CC	Shrub
Embelia caulialata	Primulaceae	CC	Vine
Niemeyera prunifera	Sapotaceae	CC	Shrub to tree
Palaquium galactoxylum	Sapotaceae	CC	Tree
Pouteria obovoidea	Sapotaceae	CC	Tree
Pouteria xerocarpa	Sapotaceae	CC	Tree
Diospyros humilis	Ebenaceae	FM	Tree
Pouteria cotinifolia	Sapotaceae	FM	Shrub
Cyathodes glauca	Ericaceae	MF	Shrub
Gaultheria hispida	Ericaceae	MF	Shrub
Leptecophylla juniperina	Ericaceae	MF	Shrub
Trochocarpa gunnii	Ericaceae	MF	Shrub
6. Fagales-Cucurbitales			
Neoalsomitra clavigera	Cucurbitaceae	CC	Vine
Diplocyclos palmatus	Cucurbitaceae	FM	Vine
Nothofagus cunninghamii	Fagaceae	MF	Tree

7. Urticaceae-Moraceae			
Dendrocnide moroides	Urticaceae	CC	Tree
Ficus congesta	Moraceae	CC	Tree
Ficus hispida	Moraceae	CC	Tree
Ficus pantoniana	Moraceae	CC	Tree
Ficus septica	Moraceae	CC	Tree
Ficus variegata	Moraceae	CC	Tree
Trophis scandens	Moraceae	CC	Vine
Ficus rubiginosa	Moraceae	FM	Tree
Urtica incisa	Urticaceae	MF	Herb
8. Rhamnaceae (ziziphoid group)			
Emmenosperma cunninghamii	Rhamnaceae	CC	Tree
Alphitonia excelsa	Rhamnaceae	FM	Tree
Pomaderris apetala	Rhamnaceae	MF	Tree to shrub
9. Fabaceae			
Austrosteensia blackii	Fabaceae	CC	Vine
Caesalpinia traceyi	Fabaceae	CC	Vine
Castanospermum australe	Fabaceae	CC	Tree
Entada phaseoloides	Fabaceae	CC	Vine
Acacia disparrima	Fabaceae	FM	Tree
Senna gaudichaudii	Fabaceae	FM	Herb to subshrub
Acacia dealbata	Fabaceae	MF	Tree
Acacia melanoxylon	Fabaceae	MF	Tree
Acacia vernicifl ua	Fabaceae	MF	Tree
10. Malpighiales-Oxalidales			
Argyrodendron peralatum	Euphorbiaceae	CC	Tree
Casearia costulata	Salicaceae	CC	Shrub
Claoxylon hillii	Euphorbiaceae	CC	Tree
Cleistanthus myrianthus	Phyllanthaceae	CC	Shrub
Elaeocarpus angustifolius	Elaeocarpaceae	CC	Tree
Garcinia warrenii	Clusiaceae	CC	Tree
Gillbeea whypallana	Cunoniaceae	CC	Tree
Glochidion sumatranum	Euphorbiaceae	CC	Tree
Mallotus paniculatus	Euphorbiaceae	CC	Tree
Passiflora kuranda	Passifloraceae	CC	Vine
Planchonella myrsinodendron	Ochnaceae	CC	Tree
Rockinghamia angustifolia	Euphorbiaceae	CC	Tree
Rourea brachyandra	Connaraceae	CC	Vine
Antidesma parvifolium	Euphorbiaceae	FM	Shrub
Breynia cernua	Euphorbiaceae	FM	Shrub
Bridelia leichhardtii	Euphorbiaceae	FM	Tree
Claoxylon tenerifolium	Euphorbiaceae	FM	Tree
Drypetes deplanchei	Euphorbiaceae	FM	Tree
Erythroxylum australe	Erythroxylaceae	FM	Shrub to tree
Flueggea leucopyrus	Euphorbiaceae	FM	Shrub
Phyllanthus tenellus	Euphorbiaceae	FM	Herb
Anodopetalum biglandulosum	Cunoniaceae	MF	Shrub
Aristotelia peduncularis	Elaeocarpaceae	MF	Shrub to vine
11. Rutaceae			
Brombya platynema	Rutaceae	CC	Tree
Flindersia bourjotiana	Rutaceae	CC	Tree
Medicosma fareana	Rutaceae	CC	Tree
Melicope xanthoxyloides	Rutaceae	CC	Tree
Geijera salicifolia	Rutaceae	FM	Tree
Nematolepis squamea	Rutaceae	MF	Tree

Zieria arborescens	Rutaceae	MF	Shrub to tree
	12. Malvales		
Sterculia quadrifida	Malvaceae	CC	Tree
Abutilon auritum	Malvaceae	FM	Shrub
Abutilon oxycarpum	Malvaceae	FM	Shrub
Brachychiton australis	Malvaceae	FM	Tree
Grewia papuana	Malvaceae	FM	Shrub
Pimelea drupacea	Thymelaeaceae	MF	Shrub
	13. Myrtaceae		
Acmena graveolens	Myrtaceae	CC	Tree
Decaspermum humile	Myrtaceae	CC	Tree
Pilidiostigma recurvum	Myrtaceae	CC	Shrub to tree
Syzygium monospermum	Myrtaceae	CC	Tree
Syzygium sayeri	Myrtaceae	CC	Tree
Gossia bidwillii	Myrtaceae	FM	Tree
Eucalyptus delegatensis	Myrtaceae	MF	Shrub
Eucalyptus obliqua	Myrtaceae	MF	Tree
Eucalyptus regnans	Myrtaceae	MF	Tree
	14. Ranunculales		
Carronia protensa	Menispermaceae	CC	Vine
Hypserpa laurina	Menispermaceae	CC	Vine
Clematis pickeringii	Ranunculaceae	FM	Vine
Tinospora smilacina	Menispermaceae	FM	Vine
Clematis aristata	Ranunculaceae	MF	Vine
	15. Poales		
Flagellaria indica	Flagellariaceae	CC	Vine
Oplismenus aemulus	Poaceae	CC	Herb
Ancistrachne uncinulata	Poaceae	FM	Herb
Cyperus bowmanii	Cyperaceae	FM	Herb
Cyperus gracilis	Cyperaceae	FM	Herb
Gahnia grandis	Cyperaceae	MF	Herb
	16. Asparagales		
Cordyline cannifolia	Asparagaceae	CC	Herb
Dendrobium linguiforme	Orchidaceae	FM	Epiphyte
Habenaria hymenophylla	Orchidaceae	FM	Herb
Dianella caerulea	Xanthorrhoeaceae	FM	Herb
Drymophila cyanocarpa	Asparagaceae	MF	Herb
Chiloglottis gunnii	Orchidaceae	MF	Herb
Corybas sp.	Orchidaceae	MF	Herb
	17. Magnoliales-Laurales		
Beilschmiedia bancroftii	Lauraceae	CC	Tree
Cryptocarya grandis	Lauraceae	CC	Tree
Cryptocarya hypospodia	Lauraceae	CC	Tree
Cryptocarya laevigata	Lauraceae	CC	Tree
Cryptocarya mackinnoniana	Lauraceae	CC	Tree
Cryptocarya murrayi	Lauraceae	CC	Tree
Doryphora aromatica	Monimiaceae	CC	Tree
Endiandra hypotephra	Lauraceae	CC	Tree
Endiandra leptodendron	Lauraceae	CC	Tree
Endiandra microneura	Lauraceae	CC	Tree
Eupomatia laurina	Eupomatiaceae	CC	Shrub
Haplostichanthus ramiflorus	Annonaceae	CC	Shrub
Hernandia albiflora	Hernandiaceae	CC	Shrub to tree
Litsea leefiana	Lauraceae	CC	Tree
Myristica globosa	Myristicaceae	CC	Tree

Palmeria scandens	Monimiaceae	CC	Vine
Pseuduvaria froggattii	Annonaceae	CC	Shrub
Steganthera laxiflora	Monimiaceae	CC	Tree
Melodorum leichhardtii	Annonaceae	FM	Tree
Atherosperma moschatum	Monimiaceae	MF	Tree
18. Polypodiaceae			
Colysis ampla	Polypodiaceae	CC	Epiphyte to vine
Grammitis billardierei	Polypodiaceae	MF	Epiphyte
Microsorum pustulatum	Polypodiaceae	MF	Epiphyte to vine
Pyrrosia rupestris	Polypodiaceae	FM	Epiphyte