Environmental and Evolutionary Influences of Light on Fern Leaf Morphology in California

Lindsey P. Agnew

ABSTRACT

Climate change is predicted to increase the amount of solar radiation reaching understory plant communities in California. The degree to which plants are able to respond to this change depends on a combination of factors, including their evolutionary history and phenotypic plasticity. Examining the phylogenetic and environmental factors affecting leaf morphology in ferns can help us predict how California's flora will respond to this environmental change. I studied environmental influences by comparing the division of four fern species to the light intensities of their habitats in northern and southern regions of the state. I investigated evolutionary influences by comparing leaf division and average habitat light intensity across the phylogeny of polypod ferns in California. Differences in light intensity along the state's latitudinal gradient caused more significant changes to leaf morphology than shading, with the morphological response varying by species. Phylogenetic analyses reveal that leaf division and habitat light intensity are highly labile traits with no strong phylogenetic signal. Lack of correlation between these traits indicates that different morphologies serve as adaptations to the same light conditions. The high phenotypic variability found in ferns suggests that they will likely be able to persist amongst the rapid environmental changes predicted for California. These findings show the importance of considering both environmental and evolutionary history when planning conservation efforts for the state's flora.

KEYWORDS

phylogenetic signal, phenotypic plasticity, leaf division, Polypodiales, pteridophytes

INTRODUCTION

Climate change is predicted to have a profound effect upon the light that reaches terrestrial vegetation. Global warming simulations specifically predict that more sunlight may reach the earth's surface in the next decades as a result of reduced amounts of cloud cover (Clement et al. 2009). In California, a warmer and dryer climate is expected to promote the expansion of grasslands and shrub lands at the expense of forests (Hayhoe et al. 2004, Cornwell et al. 2012). This "opening" of habitats will result in more light reaching understory species that may have been previously shaded. To persist in habitats that change, plants must adapt through natural selection, migrate to follow the prior conditions, adjust to the new conditions through changing their morphology or altering their life history, or some combination of these adaptations.

Given the trajectory of climate and habitat change, adjusting phenotypically to the new conditions may be the most immediately viable option for many plants (Nicotra et al. 2010). In the context of imminent climate change, it is critical to study phenotypic plasticity: the ability of an organism to respond to environmental changes by altering its physiology or morphology (Schlichting 1986). The extent of phenotypic plasticity in response to light can be dramatic. For example, the herb *Persicaria maculosa* (Polygonaceae) maximizes its photosynthetic capacity by producing 100 times more leaves with a six times smaller area to mass ratio in high light versus low light conditions (Sultan and Bazzaz 1993). Despite the importance of phenotypic plasticity, little is known about the role evolutionary history plays in constraining it (Valladares and Gianoli 2007). Understanding the phylogenetic components of phenotypic plasticity is necessary to predict trends of how plants will respond to changing light intensities.

The habitat of a plant often determines which leaf morphologies are beneficial, as different morphologies can conserve limited resources or dissipate those in excess. Across species and individuals, leaves of plants in high light environments are typically thicker and held more vertically than those of plants in low light environments (Fetcher et al. 1983). Similar trends appear within an individual: leaves exposed to full sun tend to be smaller, thicker, and heavier than leaves growing in shade (Anderson 1955, Niinemets 1998). These trends are generally a result of leaves developing extra layers of palisade mesophyll or longer palisade cells to protect themselves from damage from excess light (Xu et al. 2009). Having compound leaves is a different strategy used by some species to survive in high light conditions. Woody dicots with compound leaves tend to

occur in areas of high temperatures and low rainfall, conditions typically indicative of high light (Stowe and Brown, 1981). This pattern varies by taxa and by leaf trait: the Leguminosae and Rosaceae typically have smaller leaflets and occur in drier regions than the Juglandaceae, which typically have larger leaflets and inhabit wetter regions (Stowe and Brown, 1981). This variability indicates the need for further investigation of the phylogenetic and biogeographic factors regulating phenotypic plasticity of leaf morphology in compound-leaved species.

Ferns act as a model system for studying the phenotypic plasticity of leaf morphology in the context light intensity. In ferns each frond is a single leaf, eliminating the confounding variables associated with branching. Furthermore, fronds vary immensely in division and form both within and across species, and evolutionary history may play a role in determining the level of plasticity within individual fern groups. However, the extent of phenotypic plasticity in response to light intensity appears to vary significantly even between closely related species. Several *Adiantum* species have higher specific leaf area when grown at low light, while the plasticity of leaf area and leaf area ratio are highly species dependent (Liao et al. 2013). Similarly, Blechnaceae species differ significantly in the range of light environments occupied and in the plasticity of specific leaf area to light availability (Saldana et al. 2005). Although prior studies indicate that environmental factors play an important role in determining leaf form, this role remains unknown. A study conducted across the entire fern phylogeny is necessary to elucidate the nature of the evolutionary component to plasticity.

The purpose of this study was to determine how light intensity affects the leaf morphology of California ferns. My study quantified the relative strength of the phylogenetic and environmental factors affecting fern leaf morphology and determined which morphologies are most common under various light intensities. I hypothesized (1) ferns with smaller, thicker, and more highly-divided leaves will be found in environments with higher light intensities, and (2) environmental conditions are stronger predictors of trends in leaf morphology than phylogenetic relationships. To understand the plasticity of leaf morphological characteristics alongside light conditions, I conducted a field study comparing morphologies of individuals of three focal species to the light intensities they grew in. Additionally, I investigated the relative strength of environmental and genetic influences on morphology through a database study comparing phylogenetic relationships of polypod ferns in California to solar radiation received throughout each species' range.

METHODS

Study system

The large variety of habitats in California supports a high diversity of ferns for a non-tropical region (REF). California is home to 104 species of native ferns, nested within 32 genera and 14 families (Jepson eFlora). My field study focused on three focal species: *Polystichum munitum* (western sword fern, Dryopteridaceae), *Dryopteris arguta* (coastal woodfern, Dryopteridaceae), and *Pteridium aquilinum* (eagle fern, Dennstaedtiaceae). *Polystichum munitum* is once-pinnate, *D. arguta* is twice pinnate, and *P. aquilinum* is thrice-pinnate (Figure 1). These species are native to California, evergreen, and commonly found throughout the state (Calflora 2015). My database study included analysis of the 66 species of polypod ferns (Polypodiales) native to California.

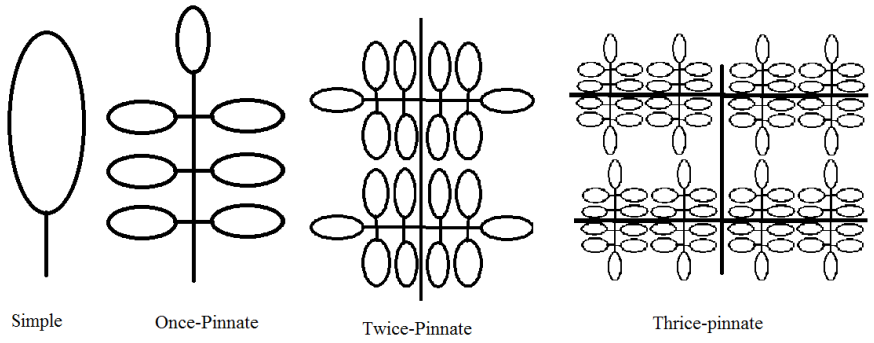

Figure 1. Illustration of pinnateness categories. Leaves were categorized and scored based on their degree of division.

Environmental influences

Data collection

To investigate how latitudinal differences in solar radiation affect leaf morphology, I collected samples of the three focal species from southern California and northern California. *Polystichum munitum* could not be located in sufficient quantity within San Diego County; instead,

I sampled *Nephrolepis cordifolia*, a non-native once-pinnate fern (Table 1). To minimize environmental differences other than incident sunlight, I selected sampling areas in similar habitat types within northern and southern California. I sampled *P. aquilinum* in inland mountainous areas of high elevation, *D. arguta* in semi-coastal, riparian hillsides, and *P. munitum* and *N. cordifolia.* in coastal, wooded slopes (Table 2, Figure 2).

Table 1. Sampling species and locations. *Nephrolepis cordifolia* was only sampled in southern California and *P. munitum* was only sampled in northern California.

Degrees of Pinnation	Species	Southern California	Northern California
Once-Pinnate	*Polystichum munitum*		X
	Nephrolepis cordifolia	X	
Twice-Pinnate	*Dryopteris arguta*	X	X
Thrice-Pinnate	*Pteridium aquilinum*	X	X

Table 2. Site descriptions. Species were sampled in areas sharing similar environmental characteristics in northern and southern California to minimize the effect of non-light environmental differences.

Location	Species	Site	GPS Coordinates	Elevation	Aspect
Southern California	*N. cordifolia*	Palm Canyon, Balboa Park, San Diego County	N 32.730258, W 117.151026	75 m	W
	D. arguta	Elfin Forest Trail near Escondido Creek, Elfin Forest, San Diego County	N 33.086461, W 117.145065	156 m	N
	P. aquilinum	Doane Valley Road near Doane Pond, Palomar Mountain, San Diego County	N 33.338682, W 116.900866	1,424 m	NE
Northern California	*P. munitum*	Fern Creek Trail, Muir Woods, Marin County	N 37.905575, W 122.578649	81 m	SW
	D. arguta	Lower Fire Trail, Strawberry Canyon, Alameda County	N 37.871025, W 122.243013	236 m	N
	P. aquilinum	Tioga Pass Road near South Fork of Tuolumne River, Yosemite, Mariposa County	N 37.797672, W 119.721114	2,055 m	E

Figure 2. Sampling locations by species.

To establish the degree of variation both within sites in response to shading and across sites in response to latitudinal differences, I measured a variety of morphological variables for fifteen randomly selected individuals at each site. I had a total sample size of 90 individuals: 15 *N. cordifolia,* 15 *P. munitum*, 30 *D. arguta*, and 30 *P. aquilinum*. I measured micromoles of incident photosynthetically active radiation (PAR) above each fern using a LICOR-250 light meter. To account for weather effects, I took a baseline reading in each area in an open, unshaded space. I then divided the light readings above each fern by the baseline reading to obtain a percentage of the available light incident on the fern. I randomly selected, photographed, and removed one frond from each of the fifteen plants. I measured the frond's length from the base to tip of the leaf blade, and the width of the leaf blade at its widest point. I measured the fresh weight of the frond using an AWS Chrome digital pocket scale. I stored leaves in plastic bags and measured the dry weight of the fronds after one minute and two minutes of microwaving (Marur and Sodek 1995).

Data analysis

To obtain descriptive values for leaf morphology, I used ImageJ software to trace the field photographed fronds and generate values for leaf area and perimeter (Rasband 2015). I calculated

values for leaf compoundness, Specific Leaf Area (SLA), and Leaf Mass per Area (LMA) for each frond. To calculate leaf compoundness, I divided leaf perimeter by the square root of leaf area. To calculate Specific Leaf Area, I divided the leaf area by its dry mass. Finally, to calculate Leaf Mass per Area I subtracted dry mass from fresh mass and divided by fresh mass.

To statistically analyze morphological response to light intensity for each species of fern sampled, I ran t-tests across sites and linear regressions within sites. Results were analyzed in R (R Development Core Team 2015). All data was tested for normality using the Shapiro-Wilks test. If data was non-normally distributed, it was log-transformed; all log-transformed data met normality requirements. To determine morphological in response to differences across sites, i.e. due to latitudinal variation, I ran a Welch two sample t-test for each of morphological variable for the two species sampled in both southern and northern California. Perimeter, area, wet weight, compoundness, SLA, and LMA were tested. To analyze morphological differences in response to differences in light received within sites, i.e. due to shading, I ran linear regressions with percent of available light received as the explanatory variable and perimeter, area, wet weight, compoundness, SLA, and LMA as the response variables.

Evolutionary influences

Phylogeny creation

To create the phylogeny, I downloaded sequences from GenBank for sequences for the following genes: maturase K (matK), ribulose bisphosphate carboxylase large chain (rbcL), ribosomal protein small subunit 4 (rps4), ATP synthase alpha chain (atpA), and ATP synthase beta chain (atpB) (Bensen et al. 2008, Table 3). Of the 65 Polypodiales ferns in California, there were sequences available for 59 species in at least one of the five genes I studied. I used *Osmunda regalis* (Osmundaceae) as an outgroup for this study. I aligned the sequences for each gene in MAFFT v7.272 (Katoh 2013). I visually inspected alignments for accuracy using AliView v.1 (Larsson 2014). I concatenated all five genes into a single sequence for each species using SequenceMatrix v1.8 (Vaidya et al. 2011). Using PartitionFinder v1.1.1 (Lanfear et al. 2012), I ran a partition analysis for each of the three codon positions for each of the five genes. I found five partitions which I included in my analysis (Table 4).

Table 4. Subset partitions for models of molecular evolution. An analysis using PartitionFinder selected five models of evolution to represent different subsets of the genes studied.

	Subset Partitions														
	atpA			atpB			matK			rbcL			rps4		
Best Model	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
TrN+I+G	✓			✓											
GTR+I+G		✓	✓		✓	✓	✓	✓		✓	✓		✓		
TVM+G									✓						
TIMef+I+G												✓			
HKY+G														✓	✓

I experimented with creating a phylogeny using three different methods, but only used the maximum likelihood phylogeny for my character state analysis. Using PAUP* v.4 (Swofford 2001), I created a neighbor joining tree. I also used PAUP* to create a parsimony-based tree. Incorporating the results of the partition analysis, I made a maximum likelihood tree using GARLI v0.951 (Zwickl 2006). Support for the parsimony and maximum likelihood analyses were evaluated using bootstrap analyses in PAUP* and GARLI with 50 repetitions.

I used divergence estimates from Pryer and Schuettpelz's (2009) timetree of ferns to time calibrate my maximum likelihood-generated tree, using estimates at three nodes (Table 5). I time calibrated the branch lengths using these estimates using the BEAUti and BEAST v.2.3.2 package with a MCMC chain length of 1,000,000 (Drummond et al. 2012).

Table 5. Divergence estimates used as priors to time calibrate the maximum likelihood tree.

Group	Age (Mya)	SD	Reference
Pteridaceae	110.80	11.08	Pryer and Schuettpetz (2009)
Eupolypods II	103.10	10.31	Pryer and Schuettpetz (2009)
Eupolypods I	98.90	9.89	Pryer and Schuettpetz (2009)

Character coding

I examined the characters of leaf division and average habitat light intensity across the phylogeny I created. To generate the morphological data, I sorted each species into pinnation category reflecting the maximum degree of pinnation their leaves display, according to the Jepson eFlora (Jepson Flora Project 2016). The general categories, with their scores indicated in parentheses, were: simple (0), unipinnate (1), bipinnate (2), tripinnate (3), and tetrapinnate (4). Decimals were added to these scorings to reflect the degree to which margins were divided: slightly lobed (.25), lobed (.5), and pinnatisect (.75).

To generate the light intensity characters, I linked solar and species distribution data on a county-wide scale. For solar data, I used annual average direct normal insolation (DNI), a $kWh/m^2/day$ measure of the solar irradiance striking a surface normal to the line of sight of the sun. I obtained a single county-wide DNI estimate by averaging the values of all of the $10km^2$ grid cells occurring in each of the 58 counties in California, using the National Renewable Energy Laboratory's Solar Prospector Tool (Solar Prospector 2016). For species distribution data, I recorded the number of species collected and accessioned in the California Consortium of Herbaria database within each of the 58 counties in California via the CalFlora database (CalFlora 2016). I obtained a single average light intensity estimate for the habitat of each species by taking an average of the light values of each county, weighted by the number of collections in that county.

Ancestral state reconstruction and phylogenetic signal tests

I mapped the traits of leaf division and light intensity onto my time-calibrated maximum likelihood phylogeny in R v3.2.1 (R Development Core Team. 2015.). I chose to use the maximum likelihood tree instead of the parsimony and neighbor joining trees because this method allowed me to explore different models of molecular evolution. I used the contMap function within the phytools package to map these traits onto my tree and reconstruct the ancestral states (Revell 2012).

I ran a suite of tests for correlation and phylogenetic signal on these traits in R. I began by assessing significance irrespective of phylogeny using a correlation test. I conducted phylogenetic independent contrasts for the traits and assessed significance through a correlation table, using the PIC function within the APE package (Paradis et al. 2004) and the cor.table function within the Picante package (Kembel et al. 2010). I conducted three tests of phylogenetic signal: Moran's I

using the abouheif.moran function within adephylo (Jombart and Dray 2008), Paegal's lambda using the phylosig function in phytools (Revell 2012), and Blomberg's K using the phylosig function in phytools (Revell 2012).

RESULTS

Environmental study

Comparisons between regions

I found significant differences between northern and southern California sites for all of the morphological variables I measured for *P. aquilinum* and most of the variables I measured for *D. arguta.* For *P. aquilinum,* the morphological variables of area, compoundness, and wet leaf weight were significantly higher in the southern California site than the northern California site (Figure 3, Table 6). The variables of perimeter, SLA (specific leaf area), and LMA were significantly higher in the northern California site than the southern California site for *P. aquilinum* (Figure 3, Table 6). For *D. arguta*, the variables of perimeter, LMA, and compoundness were significantly higher in the northern California site than in the southern California site (Figure 4, Table 6).

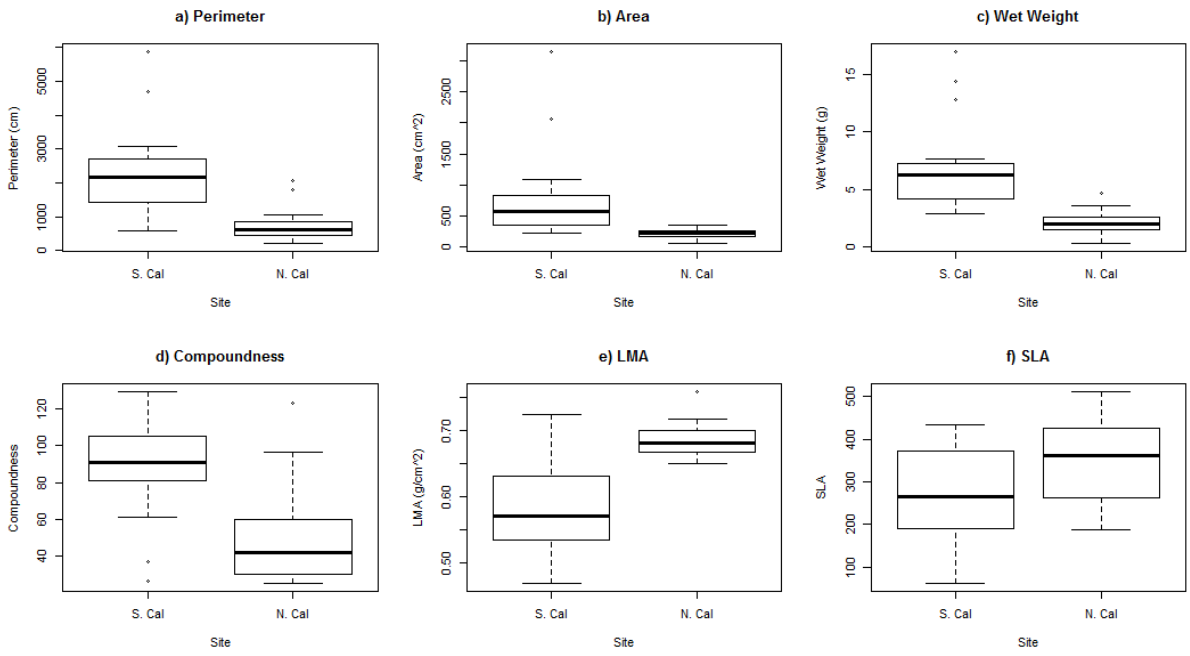

Figure 3. Differences in morphological traits of *Pteridium aquilinum* between southern and northern California sites. Leaf perimeter, area, wet weight, compoundness, LMA, and SLA were measured for all ferns sampled (n=15).

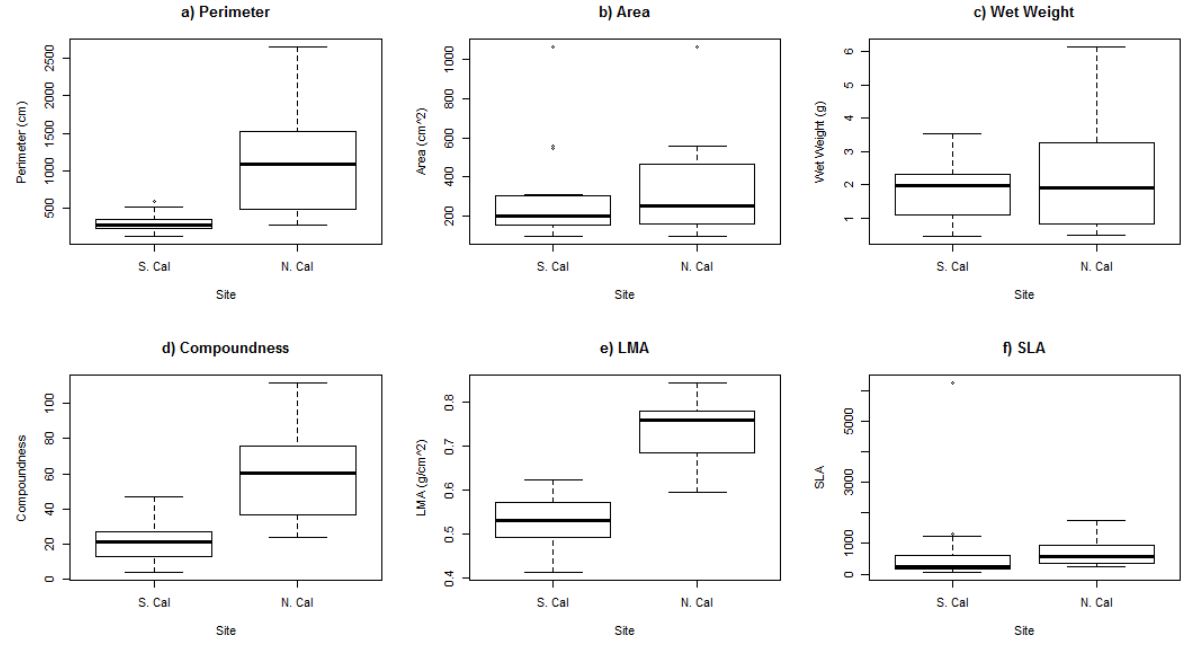

Figure 4. Differences in morphological traits of *Dryopteris arguta* between southern and northern California sites. Leaf perimeter, area, wet weight, compoundness, LMA, and SLA were measured for all ferns sampled (n=15).

Table 6. T-test results for morphological differences between northern and southern California sites. I compared species-specific data across site locations to account for latitudinal differences in solar radiation received.

Species	t	df	P
P. aquilinum			
Perimeter	5.18	28.00	0.000017*
Area	4.82	23.51	6.8×10^{-5}*
Wet Weight	5.42	27.69	9.1×10^{-6}*
Compoundness	3.58	27.65	0.0013*
LMA	-5.31	18.40	4.3×10^{-5}*
SLA	-2.23	27.97	0.034*
D. arguta			
Perimeter	-4.04	14.72	0.0011*
Area	-3.27	23.28	0.0033*
Wet Weight	-1.15	21.28	0.26
Compoundness	-5.32	19.82	3.3×10^{-5}*
LMA	-7.99	26.99	1.4×10^{-8}*
SLA	-3.04	17.17	0.0074*

Significance ($p < 0.05$) is indicated by a *.

Effect of light intensity

Light intensity differentially significantly predicted leaf morphological characteristics within each site for *P. aquilinum*. For the northern California sample of *P. aquilinum*, light intensity significantly predicted leaf compoundness ($\beta = 0.3266$, $t(13) = 5.876$, $p < 0.0001$, Figure 5) and leaf perimeter ($\beta = 0.3487$, $t(13) = 3.888$, $p < 0.01$, Figure 6). Light intensity predicted 73% of the variation in leaf compoundness ($R^2 = 0.73$, $F(1,13) = 34.53$, $p < 0.0001$) and 54% of the variation present in leaf perimeter ($R^2 = 0.54$, $F(1,13) = 15.12$, $p < 0.01$). Both variables increased with increasing light intensity. Although not significant, light intensity weakly predicted wet leaf weight ($\beta = 1.59$, $t(13) = 2.115$, $p = 0.0543$), which increased with light intensity. Although no significant trends were found in the southern California sample of *P. aquilinum*, light intensity weakly predicted LMA ($\beta = -0.11$, $t(13) = 1.919$, $p = 0.0772$), which decreased with light intensity. There were no significant correlations between leaf morphological traits and light intensity for any other of the species sampled.

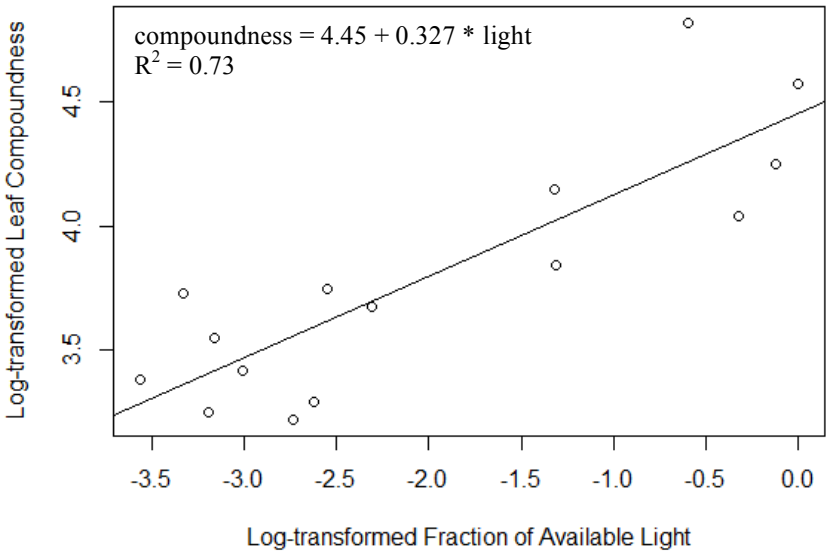

Figure 5. Response of northern California *Pteridium aquilinum* leaf compoundness to light intensity. I measured compoundness values for 15 individuals in northern California.

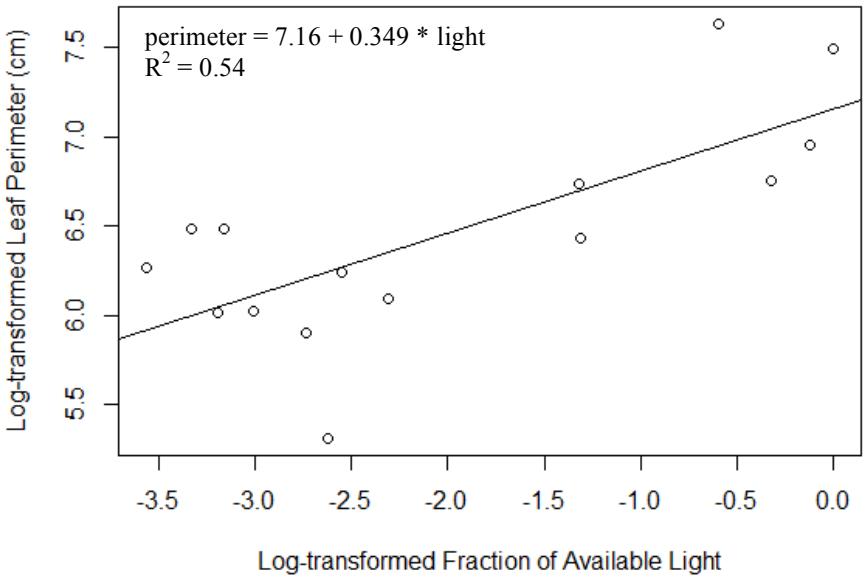

Figure 6. Response of northern California *Pteridium aquilinum* leaf perimeter to light intensity. I measured perimeter values for 15 individuals in northern California.

Evolutionary study

Phylogeny creation

There were several key differences between the results of the tree creating models. The neighbor joining tree (Figure 7) was the least similar to current taxonomic understanding and phylogenetic trees for Polypodiales (Rothfels et al. 2015). Differences between the neighbor joining tree and the other two trees include splitting *Myriopteris* into two clades and inserting *Pentagramma* and *Notholaena* between them, and splitting *Pellaea* into two highly separated clades; many of these placements were characterized by low bootstrap support. Both the neighbor joining and parsimony (Figure 8) trees placed *Pteridium aquilinum* as sister to the Eupolypod clade, following the current phylogenetic understanding (Rothfels et al. 2015), while the maximum likelihood tree (Figure 9) placed it as sister to all other species studied. The maximum likelihood tree placed *Astrolepis cochisensis* within *Pellaea*, while the better supported parsimony tree placed it as sister to *Pellaea*.

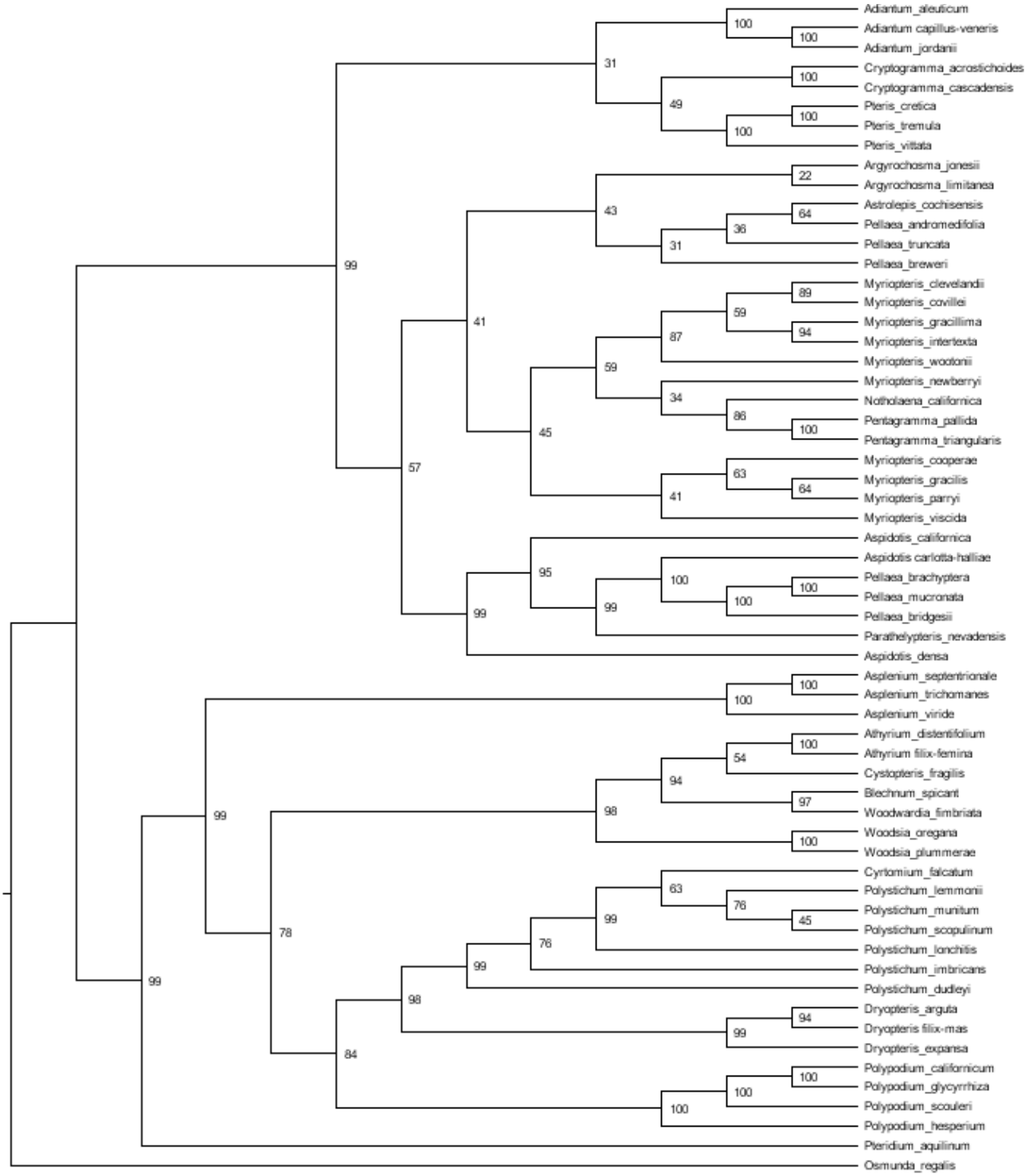

Figure 7. Neighbor joining cladogram. Bootstrap support values are indicated at nodes.

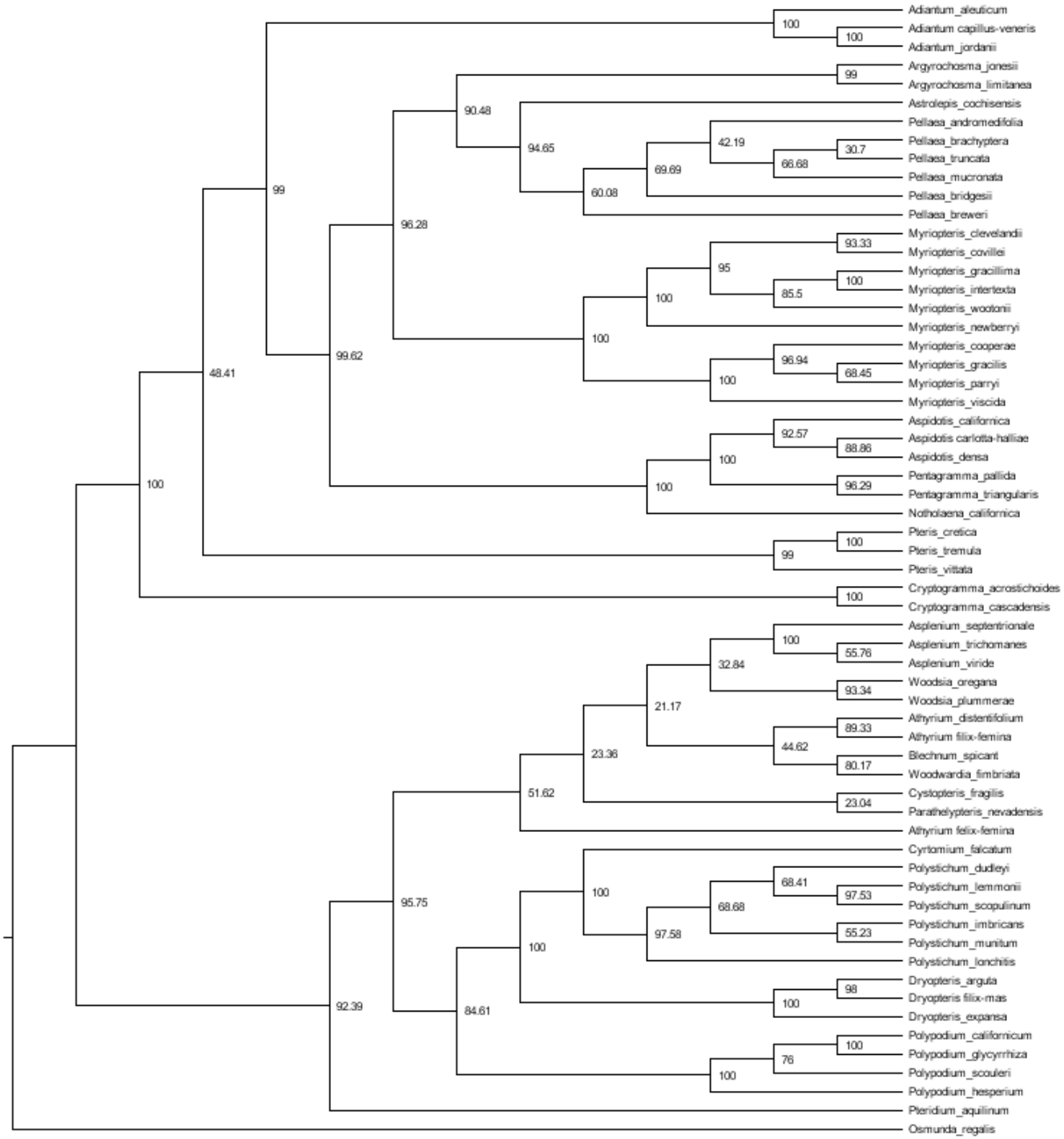

Figure 8. Parsimony tree cladogram. Bootstrap support values are indicated at nodes.

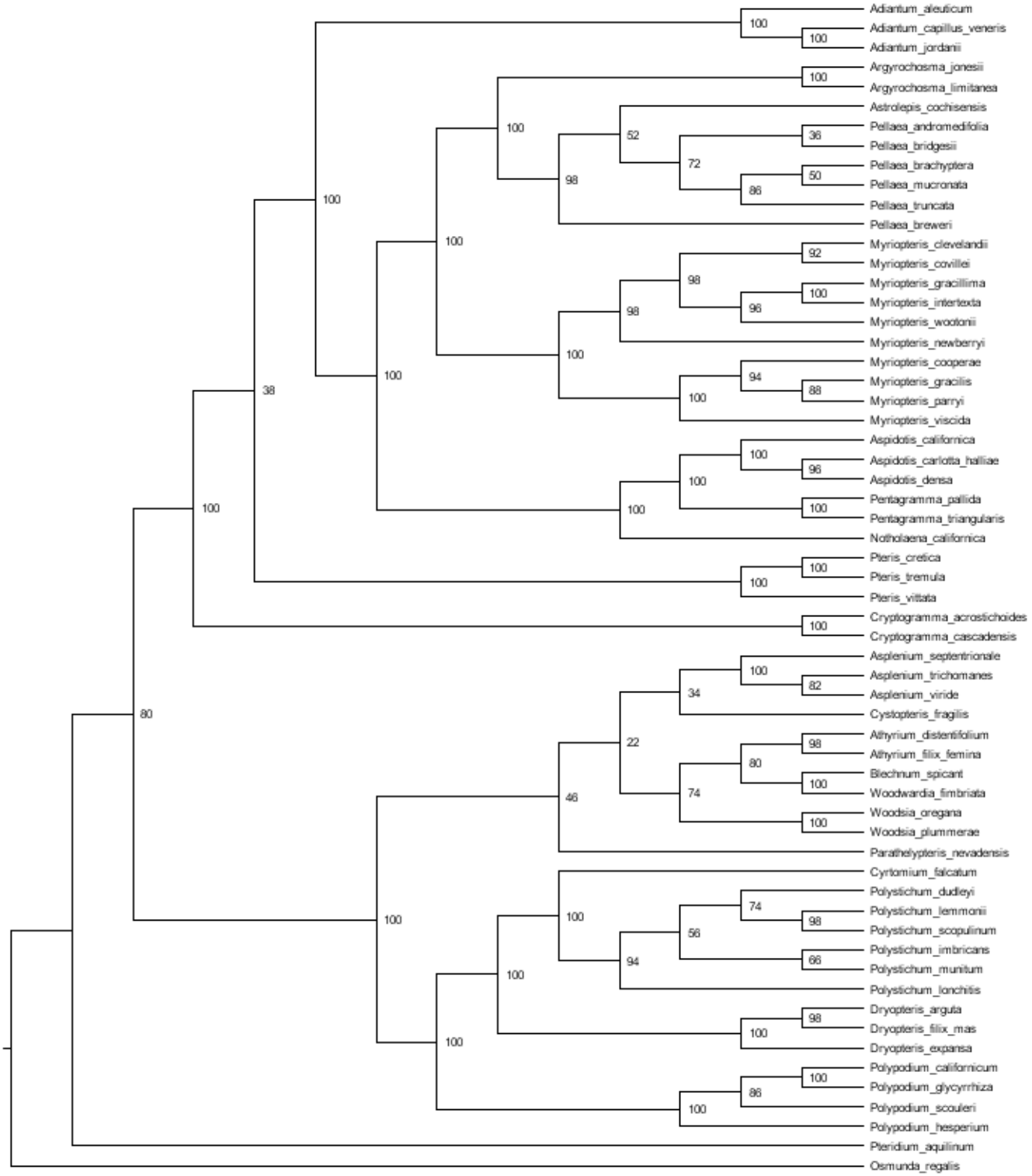

Figure 9. Maximum likelihood tree cladogram. Bootstrap support values are indicated at nodes.

The maximum likelihood trees with branch lengths representing molecular evolution (Figure 10) and historical time (Figure 11) indicate different patterns of diversification for various clades. Many genera, including *Adiantum, Athyrium, Myriopteris, Pellaea, Polystichum,* and *Woodsia*, have experienced rapid speciation with relatively low molecular diversification. The time calibrated tree shows that rapid lineage divergence occurred around 100 million years ago, followed by a period of stasis, with further diversification occurring relatively recently.

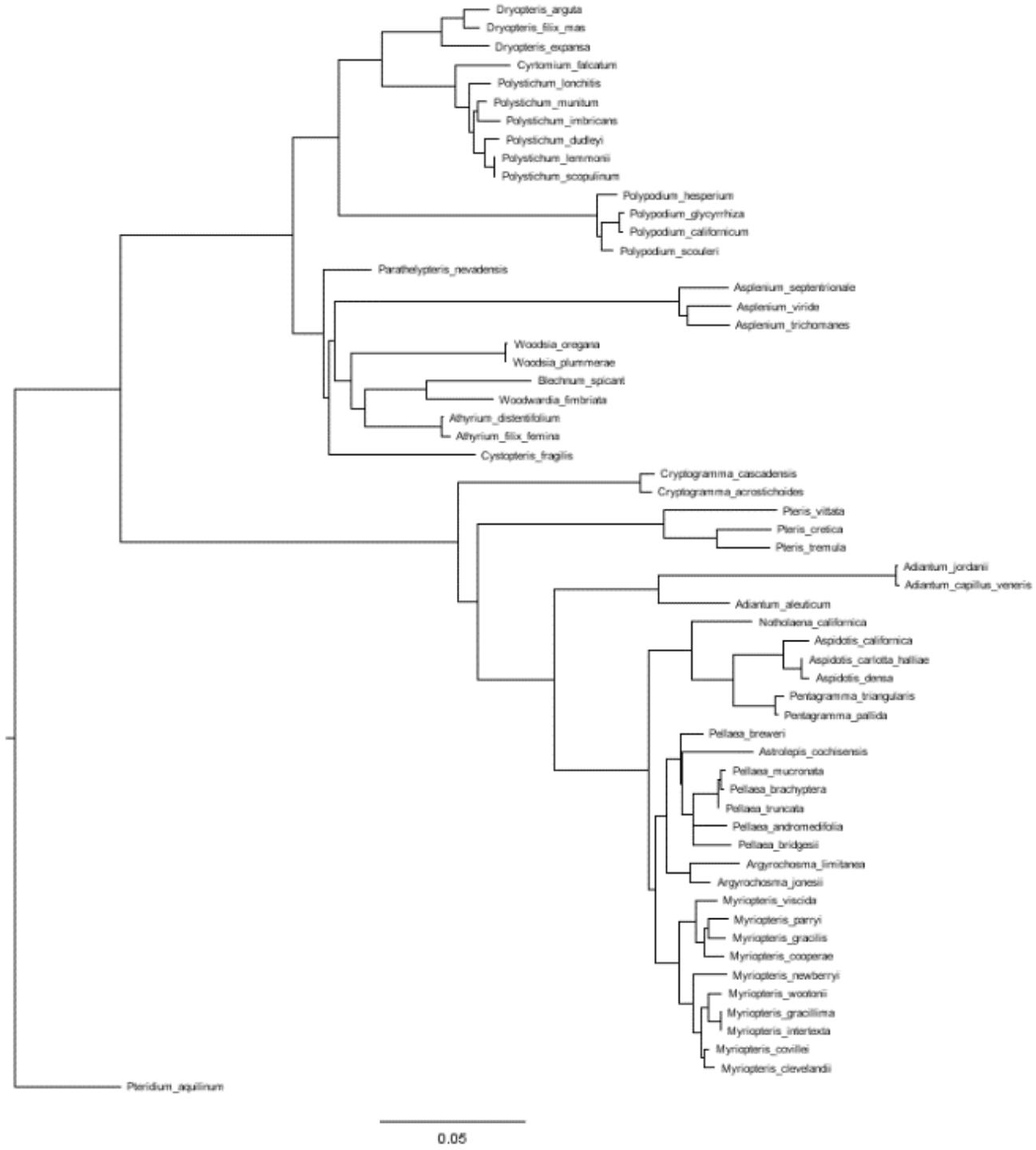

Figure 10. Maximum likelihood tree with branch lengths representing molecular evolution. The scale bar indicates branch length representing 0.05 substitutions per site.

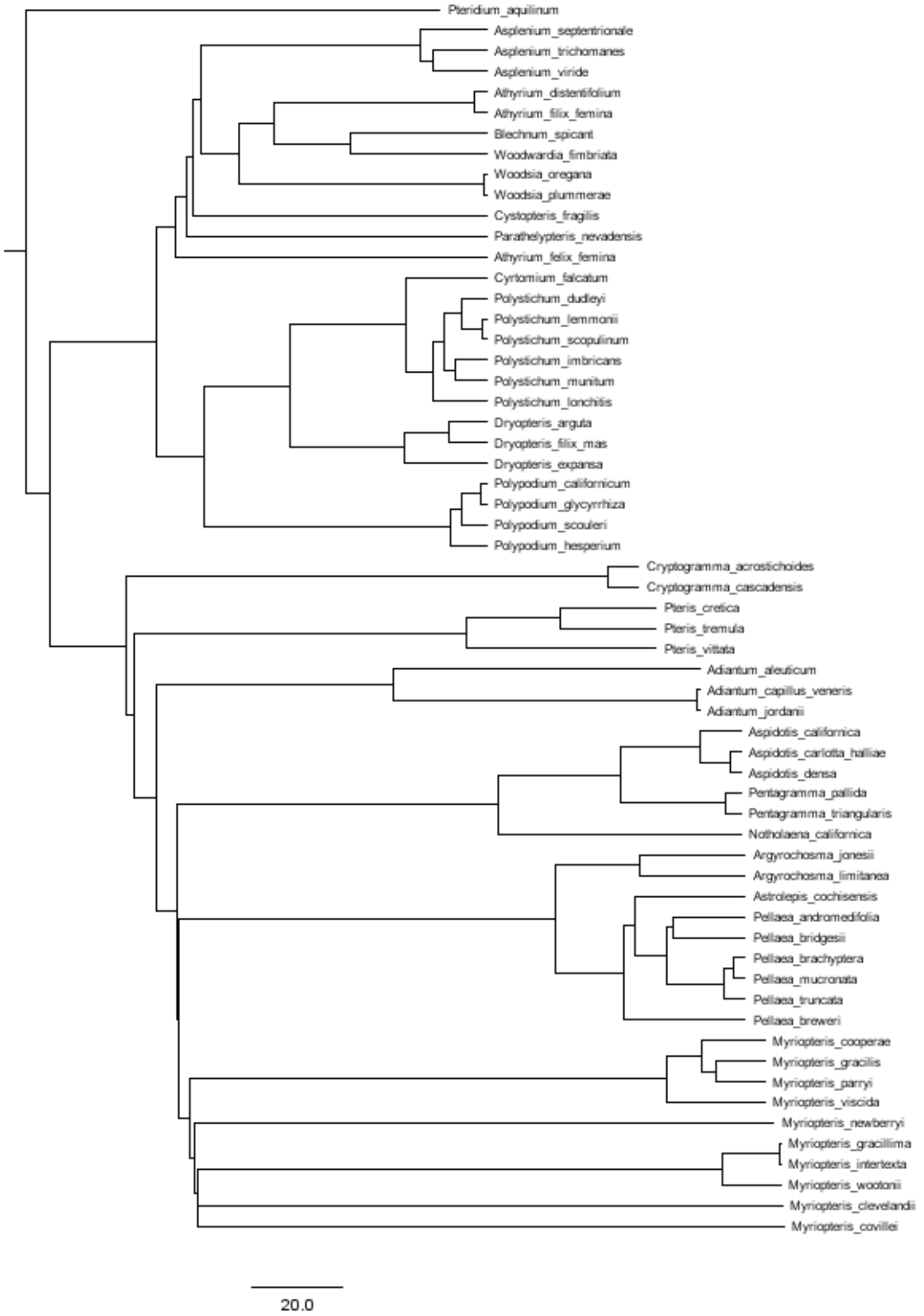

Figure 11. Maximum likelihood tree with branch lengths representing time. The scale bar indicates branch length representing a 20 million year timespan.

Character coding

The mean value ferns displayed for leaf division was 2.19, with a standard deviation of 1.02 (Table 7). *Asplenium septentrionale* was the least divided (0.25), while *Argyrochosma limitanea*, *Pallaea andromedifolia* and *Myriopteris sp.* were the most divided (4.0). The mean value ferns displayed for light intensity was 6.60 kW/m^2/day, with a standard deviation of 0.66 (Table 7). *Polypodium scouleri* received the lowest amount of radiation (5.18 kW/m^2/day), while *Astrolepis cochisensis* received the highest amount (7.92 kW/m^2/day).

Table 7. Leaf division and light intensity values, by family. SD indicates standard deviation. Light intensity is an annual average, in units of kW/m^2/day.

		Division		Light Intensity	
Family	Number of Species	Average	SD	Average	SD
Aspleniaceae	3	0.83	0.52	6.32	0.41
Blechnaceae	2	1.38	0.53	5.85	0.81
Dennstaedtiaceae	1	3.00	NA	6.45	NA
Dryopteridaceae	10	1.73	0.76	6.19	0.52
Polypodiaceae	4	1.00	0.00	6.12	0.98
Pteridaceae	32	2.63	0.99	6.81	0.60
Thelypteridaceae	1	1.75	NA	6.36	NA
Woodsiaceae	5	2.25	0.59	6.96	0.63
Total	58	2.19	1.02	6.60	0.66

Ancestral state reconstruction and phylogenetic signal tests

Trends in light intensity and leaf division varied strongly by clade. Overall, Pteridaceae tended to have more highly divided leaves than Eupolypod families, although it had the greatest standard deviation of all families examined (2.63±0.99, Table 7). Aspleniaceae displayed the least divided leaves of any family (0.83±0.52, Table 7). Woodsiaceae had the highest habitat light intensity of any family (6.69±0.63), while Blechnaceae had the lowest (5.85±0.81, Table 7). The genus *Myriopteris* tended to display high trait values for both light intensity and leaf division (Figure 12, Figure 13). *Pellaea* had mid-moderate levels of light intensity, with very mixed degrees

of leaf division (Figure 12, Figure 13). Some genera, like *Adiantum*, were in the middle of the spectra for both light intensity and leaf division (Figure 12, Figure 13).

Figure 12. Ancestral state reconstruction of degree of leaf division. Colors on the red end of the spectrum indicate less divided leaves, and colors on the blue end of the spectrum indicate more divided leaves.

Figure 13. Ancestral state reconstruction of light intensity in habitat. Colors on the red end of the spectrum indicate lower light intensities, and colors on the blue end of the spectrum indicate higher light intensities ($kW/m^2/day$).

The average light intensity of the habitat ferns occupy and the degree to which their leaves are divided are not significantly associated with each other, with or without evolutionary context.

A correlation test between habitat light intensity and leaf division revealed no significant relationship between the two traits (Figure 14). A phylogenetic independent contrast between habitat light intensity and leaf division also revealed no significant relationship (Figure 15).

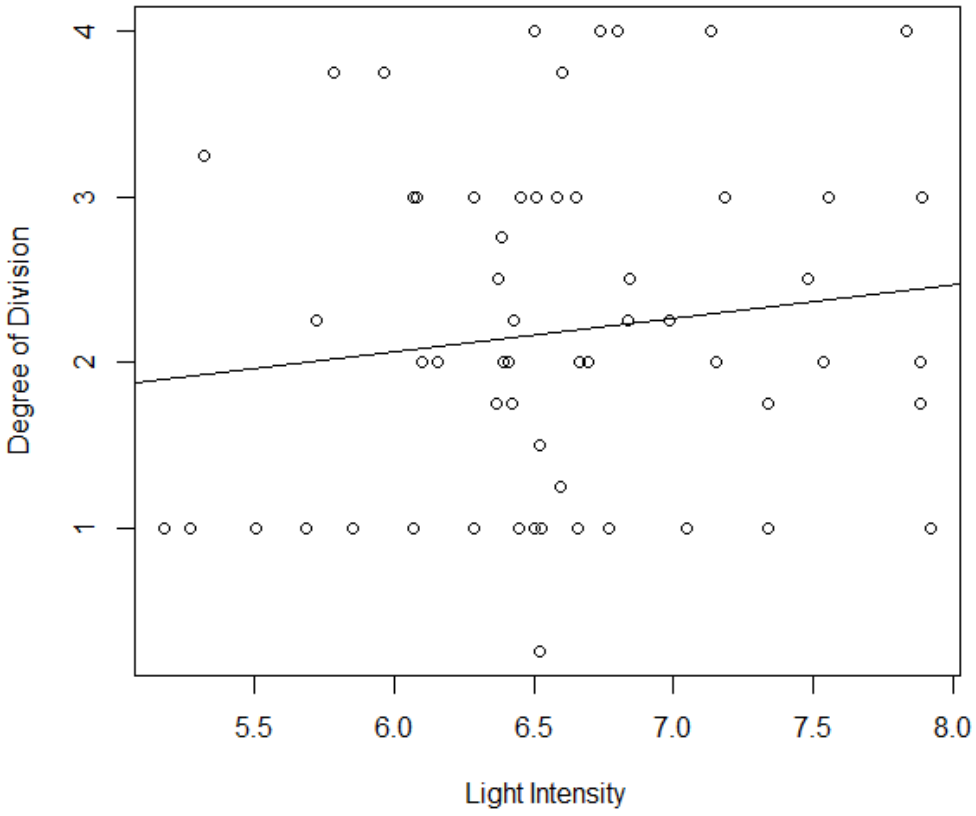

Figure 14. Scatterplot of leaf division and light intensity. No statistically significant trend is present.

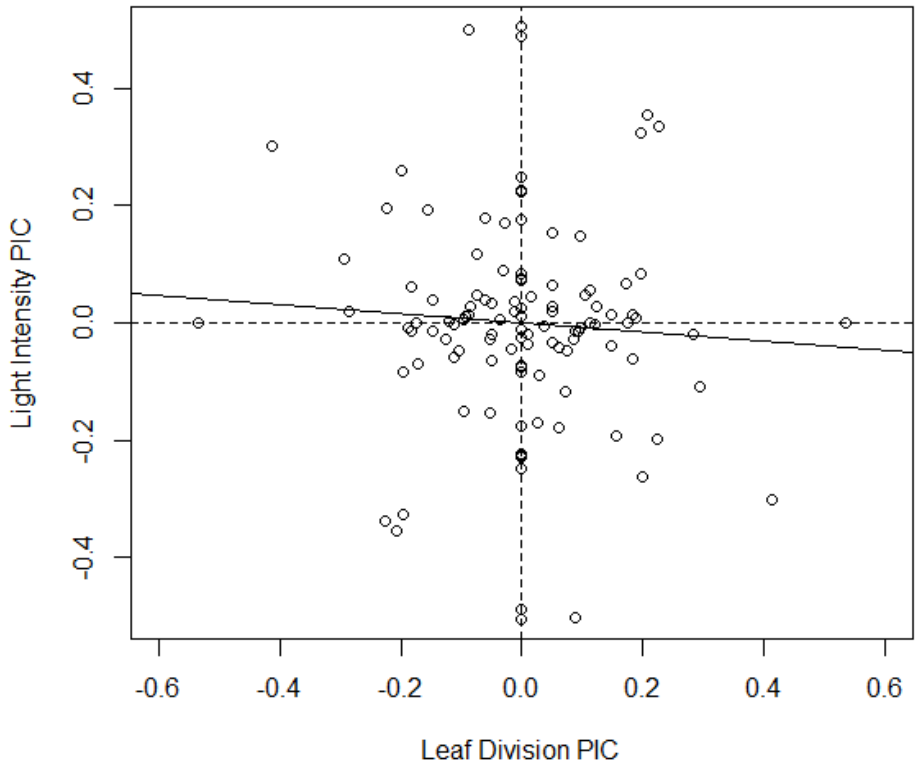

Figure 15. Phylogenetic independent contrast of leaf division and habitat light intensity. Axes represent the degree to which compared taxa share similar values for a trait. No statistically significant trend is present.

The three tests for phylogenetic signal I conducted indicate different findings. Moran's I indicates that closely related species display more similar leaf morphologies (p=0.001) and inhabit areas with more similar light intensities (p=0.007) than would be expected under Brownian motion (Figure 16). Paegal's lambda indicates that tree structure can explain changes in morphology (λ=0.99, p=6.7e-08), but not light habitat (λ=0.21, p=0.046), under Brownian motion. Blomberg's K shows that species resemble each other as much as could be expected under Brownian motion for leaf division (K=0.41, p=0.0001) and light intensity (K=0.10, p=0.036). Because the K for light intensity was far from 1, a test was run randomly resampling the data over 100 replicates and produced a mean K of 0.054. This confirmed that light intensity showed that the expected value for K under random evolution, and was not less similar than would be expected at random.

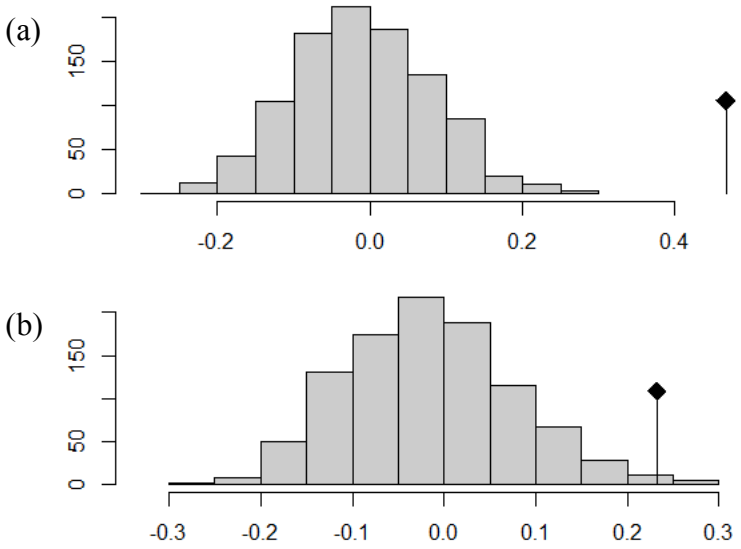

Figure 16. Moran's I histograms. Plots are shown for leaf division (a) and light intensity (b).

DISCUSSION

Large-scale factors, both environmental and evolutionary in nature, may play a greater role in determining leaf morphology than fine-scale factors. The results of this study suggest that latitudinal differences in solar radiation more significantly affect leaf morphology than small-scale factors such as overstory shading. The lack of strong phylogenetic signal for habitat light intensity and leaf division, despite some trends that appeared at a broader taxonomic level, may indicate the significance of more basal lineage divergences over more recent divergences. The labile nature of leaf division and habitat light intensity suggests ferns may respond positively to future environmental change. These findings show the importance of considering the strength of environmental and evolutionary influences when creating taxa-based conservation plans.

Environmental influence

Large-scale environmental conditions affecting incident light play a strong role in determining leaf morphology across all species, but may differentially affect species with highly divided leaves. The differential responses of twice-pinnate *D. arguta* and thrice-pinnate *P. aquilinum* in response to latitudinal light gradients could relate back to the nature of compound leaves. While little research has been done into the environmental correlations of varying pinnation

levels, many comparative studies between compound and simple leaves have been conducted. Compound leaves share fundamental developmental similarities with lobes on simple leaves (Sinha 1997). Because they facilitate gas exchange, toothed leaves (a type of lobed leaf) are correlated with colder environments, and entire leaves with warmer conditions (Royer and Wilf 2006). Furthermore, minor veins, of which there are many relative to primary veins in compound leaves, are highly sensitive to environmental conditions (Givnish 1979, Sack et al. 2003). The larger, heavier, and more compound *P. aquilinum* leaves found in cooler northern California could be a result of selection for similar advantages to teething in simple leaves. *D. arguta,* which had larger and more compound leaves in warmer and sunnier southern California, could have shown a different response because it has less minor veins relative to primary veins and is therefore less sensitive to environmental changes.

Although shading is not predictive of leaf morphology for all species studied except *P. aquilinum* in northern California, shading can have dramatic morphological effects on other species (Anderson 1955, Lichtenthaler 1981, Malhado et al. 2010, Niinemets 1998, Sultan and Bazzaz 1993). In contrast to large-scale environmental trends, the significance of morphology in response to light intensity in the northern California site may result from habitat conditions, not the level of division. While the southern California site displayed typical climax forest conditions, the northern California site was very open and seemed likely to be experiencing post-fire succession. It is possible that the availability of niches created by this disturbance allowed for greater variation in phenotypic expression of *P. aquilinum* within this site. Leaf heterogeneity and phenotypic plasticity has been viewed as evidence of niche differentiation in coexisting species of oaks (Xu et al. 2009) and begonias (McLellan 2000). With the opening of niches and reduction in competition in early successional stages (Pickett 1987), phenotypic heterogeneity in response to light intensity may have allowed for temporary differentiation into niches *P. aquilinum* is typically competitively excluded from.

Evolutionary influence

The lack of correlation among phylogenetic relationships, leaf division, and habitat light intensity of the ferns studied shows that these traits have extremely labile evolutionary histories. Compound leaves have independently evolved multiple times in highly differentiated plant

lineages: ferns, cycads and flowering plants; even within lineages, compound leaves are readily gained and lost (Sinha 1997). In angiosperms, compound leaves are gained at a relatively slow evolutionary rate and lost rapidly (Geeta et al. 2007). If this was the case in ferns, ancestral state reconstruction would show rate distinctions between leaves increasing and decreasing in compoundness. However, my time calibrated tree showed both long and short branch lengths corresponding to both increasing and decreasing division. This indicates that ferns have an evolutionary history of compound leaves distinct from that of angiosperms. Fern leaves have been found to function following a cantilever beam model – unique from the vertical pole model used for seed plants (Peppe et al. 2013). This model, which has altered conditions of petiole function, may be responsible for the insensitivity of fern physiognomy to environmental conditions (Peppe et al. 2013). Conversely, this lack of correlation may be due to ferns experiencing a unique subcanopy microclimate, which can be very distinct from the overall regional climate (Bailey and Sinnott 1916).

The lack of correlation between leaf division and current habitat light levels may also be evidence that current morphological trends are a reflection of past conditions, not the present environment. This hypothesis is further supported by the differential morphological trends seen in the northern and southern ranges of two of the species tested. Royer et al. (2005) have found that fossilized leaves are reliable indicators of climatic conditions during their time of growth, with leaves with fewer teeth, smaller tooth area, and less blade dissection characterizing historically warmer environments. Perhaps these ferns, often referred to as "living fossils" (Bateman et al. 1998, Bomfleur et al. 2014, Meeuse 1961), retain a signal of their historical environment in lieu of their current one. Bomfleur et al. (2014) found evidence of extreme evolutionary stasis within *Osmundastrum cinnamomea* (Osmundaceae). They compared preserved molecular data in a fossilized leaf to the fern's current genetics and found that its nuclei have remained relatively unchanged over 180 million years. Within this context, the rapid diversification of lineages I found to occur within California Polypodiales ferns circa 100 million years ago, followed by a period of stasis, should be examined to see if the climatic conditions of that time may have resulted in the morphology present today.

Limitations and next steps

Highly divided fern leaves may be highly morphologically responsive to changes in light intensity; examining this effect over a larger area, number of species, and number of individuals would elaborate upon this trend. My field study was limited in sample size, with only 15 individuals per study site and two sites per species. The once-pinnate fern cross-site comparison involved a substitution with two species in different genera, making inference difficult. Further research should increase sample size. If there is no stronger within-site trend for all species, then it confirms that the significance of within-site differences for *P. aquilinum* in northern California is unique to that plot. This finding would prompt future research into the influence that successional stage has on degree of phenotypic plasticity in ferns. Additionally, I only investigated evolutionary relationships within representatives of the Polypodiales clade currently found in California. This taxon sampling resulted in a geographically-bounded phylogenetic tree missing many lineages and may not provide a complete picture of the evolution of different leaf forms. Geographic studies of this nature may not be valid study designs if accuracy is desired for phylogenetic signal or ancestral state reconstruction analyses. Future studies should expand analysis throughout the entire global fern phylogeny and incorporate extinct lineages through fossils; this would help determine if the morphological and habitat lability I found are specific to only Polypodiales in California, or if the trends remain true across lineages, geographic regions, and time.

Broader implications and conclusion

Fern species in this study displayed extreme lability in leaf division and the light intensity of their habitats. Because of the relative evolutionary ease at which these traits evolve and revert between states, and the high responsiveness of leaves to light intensity in a recently disturbed site, California's Polypodiales ferns can be predicted to respond well to the expected future climatic changes in California. California is predicted to have an "opening" of habitats in the future, as overstory decreases in response to higher temperatures and less moisture (Hayhoe et al. 2004, Cornwell et al. 2012). This opening will result in more light reaching understory species, ferns

included. In light of my findings, no particular care needs to be taken to protect fern species while they adjust to this change. As angiosperm lineages have distinct evolutionary and morphological constraints from ferns, they may display less trait lability and may be more vulnerable to future changes of this nature. This study shows the inextricable link between environmental and evolutionary history, and the importance of considering these factors in concert with each other when predicting how species will respond to change.

ACKNOWLEDGEMENTS

I thank Dr. Patina Mendez for her continual support, detailed feedback, and positive energy throughout the thesis process; her advice was essential in my completing this project. I also thank the rest of the ESPM 175 team for their passionate instruction: Dr. Kurt Spreyer, Anne Murray, and Abby Cochran. Dr. John Game mentored me from start to finish on my thesis, giving me excellent feedback every step of the way. I also thank him for countless hours spent patiently teaching me about pteridophytes in the UC and Jepson Herbaria. Professors Brent Mishler and Carl Rothfels provided invaluable instruction in phylogenetics and advice about designing and conducting my phylogenetic study. I thank Will Freymen for his patience and skill advising me on phylogenetic methods and software. Professor John Battles gave me critical feedback on my initial study design and helped me decide my fieldwork methods. I also thank him for letting me borrow his lab's light meter. I thank my parents for their love and support throughout my undergraduate career and for being superb companions on fern-collecting hiking trips. Finally, I am immensely grateful for the friendship, passion, and support from the students in my Environmental Science cohort.

REFERENCES

Anderson, Y. O. 1955. Seasonal development in sun and shade leaves. Ecology 36:430-439.

Bailey I. W. and E. W. Sinnott. 1916. The climatic distribution of certain types of angiosperm leaves. American Journal of Botany 3:24–39.

Bateman, R. M., P. R. Crane, W. A. DiMichele, P. R. Kenrick, N. P. Rowe, T. Speck, and W. E. Stein. 1998. Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. Annual Review of Ecology and Systematics 29:263-292.

Benson D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2009. GenBank. Nucleic Acids Research 37:D26-31.

Bomfleur, B., S. McLoughlin, V. Vajda. 2014. Fossilized nuclei and chromosomes reveal 180 million years of genomic stasis in royal ferns. Science 21:1376-1377.

Calflora: Information on California plants for education, research and conservation. 2015. The Calflora Database. http://www.calflora.org/.

Clement, A., R. Burgman, and J. Norris. 2009. Observational and model evidence for positive low-level cloud feedback. Science 325:460–464.

Cornwell, W. K., S. A. Stuart, A. Ramirez, C. R. Dolanc, J. H. Thorne, and D. D. Ackerly. 2012. Climate change impacts on California vegetation: physiology, life history, and ecosystem change. UC Davis: Information Center for the Environment.

Drummond, A.J., M. A. Suchard, D. Xie and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST. Version 1.7.

Fetcher, N., B. R. Strain, and S. F. Oberbauer. 1983. Effects of light regime on the growth, leaf morphology, and water relations of seedlings of two species of tropical trees. Oecologia 58:314-319.

Geeta, R., L. M. Davalos, A. Levy, L. Bohs, M. Lavin, K. Mummenhoff, N. Sinha, and M. Wojciechowski. 2012. Keeping it simple: Flowering plants tend to retain, and revert to, simple leaves. New Phytologist 193:481-493.

Givnish, T. J. 1979. On the adaptive significance of leaf form. In Topics in plant population biology (pp. 375-407). Macmillan Education UK. Givnish, T. J. 1979. On the adaptive significance of leaf form. Pages 375-407 in O. T. Solbrig, S. Jain, G. B. Johnson, P. H. Raven (eds.) Topics in Plant Population Biology. Macmillan, London, UK.

Hayhoe, K., D. Cayan, C. B. Field, P. C. Frumhoff, E. P. Maurer, N. L. Miller, S. C. Moser, S. H. Schneider, K. N. Cahill, E. E. Cleland, L. Dale, R. Drapek, R. M. Hanemann, L. S. Kalkstein, J. Lenihan, C. K. Lunch, R. P. Neilson, S. C. Sheridan , and J. H. Verville. 2004. Emissions pathways, climate change, and impacts on California. Proceedings of the National Academy of Sciences 101:12422–12427.

Jepson Flora Project. 2015. Jepson eFlora, http://ucjeps.berkeley.edu/IJM.html.

Jombart T., Dray S. 2008. Adephylo: exploratory analyses for the phylogenetic comparative method. Bioinformatics 26:1907-1909.

Katoh, S. 2013. MAFFT multiple sequence alignment software. Version 7.0.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C.O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463-1464.

Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Version 1.0.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Version 1.1.1.

Liao, J., M. Jiang, and H. Huang. 2013. Growth characteristics of Adiantum reniforme var. sinensis and A. capillus-veneris in response to light and soil moisture. Nordic Journal of Botany 31:500-504.

Lichtenthaler, H. K., C. Buschmann, M. Döll, H.-J. Fietz, T. Bach, U. Kozel, D. Meier, and U. Rahmsdorf. 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynthesis Research 2:115-141.

Malhado, A. C. M, R. J. Whittaker, Y. Malhi, R. J. Ladle, H. ter Steege, O. Phillips, L. E. O. C. Aragão, T. R. Baker, L. Arroyo, S. Almeida, N. Higuchi, T. J. Killeen, A. Monteagudo, N. C. A. Pitman, A. Prieto, R. P. Salomão, R. Vásquez-Martínez, W. F. Laurance, and H. Ramírez-Angulo. 2010. Are compound leaves an adaptation to seasonal drought or to rapid growth? Evidence from the Amazon Rain Forest. Global Ecology and Biogeography 19:852-862.

Marur, C. J. and L. Sodek. 1995. Microwave drying of plant material for biochemical analysis. Revista Brasileira de Fisiologia Vegetal. 7:111-114.

McLellan, T. 2000. Geographic variation and plasticity of leaf shape and size in Begonia dregei and B. homonyma (Begoniaceae). Botanical Journal of the Linnean Society 132:79–95.

Meeuse, A. D. J. 1961. Marsileales and Salviniales - “living fossils”. Acta Botanica Neerlandica 10:257–260.

Nicotra, A. B., O. K. Atkin, S. P. Bonser, A. M. Davidson, E. J. Finnegan, U. Mathesius, P. Poot, M. D. Purugganan, C. L. Richards, F. Valladares, and M. van Kleunen. 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15:684-692.

Niinemets, U. 1998. Are compound-leaved woody species inherently shade-intolerant? An analysis of species ecological requirements and foliar support costs. Plant Ecology 134:1-11.

Paradis E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R

language. Bioinformatics 20:289-290.

Peppe, D. J., C. R. Lemons, D. L Royer, S. L. Wing, I. J. Wright, C. H. Lusk, and C. H. Rhoden. 2014. Biomechanical and leaf–climate relationships: a comparison of ferns and seed plants. American Journal of Botany 101:338–347.

Pickett, S. T. A., S. L. Collins &J. J. Armesto. 1987. Models, mechanisms and pathways of succession. Bot. Rev.53: 335–371.

Pryer, K.M. and E. Schuettpelz. 2009. Ferns. Pages 153-156 in S. B. Hedges and S. Kumar (eds.) Timetree of Life. Oxford University Press, New York, New York, USA.

R Development Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org/ (Version: 0.98.501).

Rasband, W. S. 2015. ImageJ. U. S. National Institutes of Health. http://imagej.nih.gov/ij/.

Revell, L. J. 2012. Phytools: An R package for phylogenetic comparative biology (and other things). Methods of Ecology and Evolution 3:217-223.

Rothfels, C. J., F. Li, E. M. Sigel, L. Huiet, A. Larsson, D. O. Burge, M. Ruhsam, M. Deyholos, D. E. Soltis, C. N. Stewart, S. W. Shaw, L. Pokorny, T. Chen, C. dePamphilis, L. DeGironimo, L. Chen, X. Wei, X. Sun, P. Korall, D. W. Stevenson, S. W. Graham, G. S. Wong, and K. M. Pryer. 2015. The evolutionary history of ferns inferred from 25 low-copy nuclear genes. American Journal of Botany 102:1-19.

Royer, D. L., P. Wilf, D. A. Janesko, E. A. Kowalski, and D. L. Dilcher. 2005. Correlations of climate and plant ecology to leaf size and shape: potential proxies for the fossil record. American Journal of Botany 92:1141-1151.

Royer, D. L. and P. Wilf. 2006. Why do toothed leaves correlate with cold climates? Gas

exchange at leaf margins provides new insights into a classic paleotemperature proxy. International Journal of Plant Sciences 167:11–18.

Sack, L., P.D. Cowan, N. Jaikumar, and N. M. Holbrook. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. Plant Cell Environment 26:1343–1356.

Saldana, A., E. Gianoli, and C. Lusk. 2005. Ecophysiological responses to light availability in three Blechnum species (Pteridophyta, Blechnaceae) of different ecological breadth. Oecologia 145:252-257.

Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17:667-693.

Sinha, N. 1997. Simple and compound leaves: reduction or multiplication? Trends in Plant Science 2:396–402.

Solar Prospector. 2016. National Renewable Energy Laboratory. http://maps.nrel.gov/prospector.

Stowe, L. G. and J. L. Brown. 1981. A geographic perspective on the ecology of compound leaves. Evolution 35:818–821.

Sultan, S. 2007. Development in context: the timely emergence of eco-devo. Trends in Ecology and Evolution 22:575-582.

Sultan S. E. and F. A. Bazzaz. 1993. Phenotypic plasticity in Polygonum persicaria: diversity and uniformity in genotypic norms of reaction to light. Evolution 47:1009-1031.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4.0.

Vaidya, G., D. J. Lohman, and R. Meier. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Version 1.8.

Valladares, F. and E. Gianoli. 2007. How much ecology do we need to know to restore Mediterranean ecosystems? Restoration Ecology 15:363-368.

Xu, F., W. Guo, W. Xu, Y. Wei, and R. Wang. 2009. Leaf morphology correlates with water and light availability: what consequences for simple and compound leaves? Progress in Natural Science 19:1789–1798.

Zwickl, D. J. 2006. GARLI: genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, Austin, Texas, USA. Version 0.951.

APPENDIX A: GenBank Accession Numbers

Table 3. GenBank accession numbers for Polypodiales ferns in California. No accession number indicates a sequence for that gene was not available for that species.

Family	Genus	Species	rbcL	matK	atpA	atpB	rps4

Aspleniaceae	*Asplenium*	*septentrionale*	KP899650.1	JF832254.1	JF832093.1	JF832152.1	AY549777.1
Aspleniaceae	*Asplenium*	*trichomanes*	EF463157.1	JF832256.1	EF463613.1	EF463349.1	EF645629.1
Aspleniaceae	*Asplenium*	*vespertinum*					
Aspleniaceae	*Asplenium*	*viride*	KF186528.1		KF186560.1	EU352267.1	AY549782.1
Blechnaceae	*Blechnum*	*spicant*	JF832059.1	JF832262.1	EF463618.1	EF463354.1	
Blechnaceae	*Woodwardia*	*fimbriata*	AB040597.1				AF533859.1
Dennstaedtiaceae	*Pteridium*	*aquilinum*	AY300097.1	FR865060.1	JF303987.1	U93835.2	GU478637.1
Dryopteridaceae	*Cyrtomium*	*falcatum*	EF463176.1	JF303945.1	EF463671.1	EF463387.1	KF020442.1
Dryopteridaceae	*Dryopteris*	*arguta*	JQ935258.1	JQ941660.1			
Dryopteridaceae	*Dryopteris*	*expansa*	EF463179.1	JQ941612.1	EF463674.1	EF463390.1	KF020440.1
Dryopteridaceae	*Dryopteris*	*filix-mas*	KF186514.1	JQ941618.1	EF463675.1	JF832164.1	HQ680978.1
Dryopteridaceae	*Polystichum*	*californicum*					
Dryopteridaceae	*Polystichum*	*dudleyi*	AF537241.1				
Dryopteridaceae	*Polystichum*	*imbricans*	AF537262.1				
Dryopteridaceae	*Polystichum*	*kruckebergii*					
Dryopteridaceae	*Polystichum*	*lemmonii*	EF177324.1		EF463721.1	EF463437.1	
Dryopteridaceae	*Polystichum*	*lonchitis*	AB575203.1				KC890813.1
Dryopteridaceae	*Polystichum*	*munitum*	JN189508.1	JQ941636.1	EF463722.1	EF463438.1	
Dryopteridaceae	*Polystichum*	*scopulinum*	KC878856.1				KC890817.1
Osmundaceae	*Osmunda*	*regalis*	AB639179.1	HF585137.1	EF588685.1	EF588726.1	EF588771.1
Polypodiaceae	*Polypodium*	*californicum*	KF909040.1	KF909014.1	KF909083.1		
Polypodiaceae	*Polypodium*	*calirhiza*					
Polypodiaceae	*Polypodium*	*glycyrrhiza*	KF909052.1	KF909021.1	KF909080.1	AY459518.1	FJ825671.1
Polypodiaceae	*Polypodium*	*hesperium*	EU352309.1			EU352282.1	
Polypodiaceae	*Polypodium*	*scouleri*	KF909059.1	KF909029.1	KF909090.1		FJ825664.1
Pteridaceae	*Adiantum*	*aleuticum*	JF935362.1		JF937320.1	JF935447.1	JF980631.1
Pteridaceae	*Adiantum*	*capillus-veneris*	DQ432659.1	NC_004766.1	JF937300.1	JF935427.1	KU147305.1
Pteridaceae	*Adiantum*	*jordanii*	JF935348.1		JF937303.1	JF935430.1	JF980614.1
Pteridaceae	*Argyrochosma*	*jonesii*	EU268772.1		HQ846405.1		DQ914126.1
Pteridaceae	*Argyrochosma*	*limitanea*	EF452139.1		EF452077.1	EF452019.1	DQ914127.1
Pteridaceae	*Aspidotis*	*californica*	JX313525.1	JX313624.1			DQ914129.1
Pteridaceae	*Aspidotis*	*carlotta-halliae*					DQ914130.1
Pteridaceae	*Aspidotis*	*densa*	EU268773.1	JX313625.1	EU268723.1		DQ914131.1
Pteridaceae	*Astrolepis*	*cochisensis*	KF289708.1	KF289578.1			
Pteridaceae	*Cryptogramma*	*acrostichoides*	KC700102.1				DQ914172.1
Pteridaceae	*Cryptogramma*	*cascadensis*	KC700087.1				KC700204.1

Pteridaceae	*Myriopteris*	*clevelandii*	KF961777.1		KF961714.1		
Pteridaceae	*Myriopteris*	*cooperae*	KF961778.1		KF961716.1		
Pteridaceae	*Myriopteris*	*covillei*	KF961779.1		KF961715.1	KC984444.1	DQ914146.1
Pteridaceae	*Myriopteris*	*gracilis*	KF961783.1		KF961720.1		EU831151.1
Pteridaceae	*Myriopteris*	*gracillima*	KF961787.1		KF961724.1		DQ914152.1
Pteridaceae	*Myriopteris*	*intertexta*	KF961790.1		KF961727.1		
Pteridaceae	*Myriopteris*	*newberryi*	EU268787.1		EU268738.1	FJ870827.1	EU831152.1
Pteridaceae	*Myriopteris*	*parryi*	KF961815.1		KF961751.1		
Pteridaceae	*Myriopteris*	*viscida*	KF961821.1		KF961757.1		
Pteridaceae	*Myriopteris*	*wootonii*	KF961823.1		KF961759.1	FJ870830.1	FJ870852.1
Pteridaceae	*Notholaena*	*californica*	EU268792.1		EU268747.1		DQ914167.1
Pteridaceae	*Pellaea*	*andromedifolia*	U19501.1				EU831109.1
Pteridaceae	*Pellaea*	*brachyptera*					EU831112.1
Pteridaceae	*Pellaea*	*breweri*	EU268808.1		EU268764.1		EU831116.1
Pteridaceae	*Pellaea*	*bridgesii*					EU831118.1
Pteridaceae	*Pellaea*	*mucronata*					EU831123.1
Pteridaceae	*Pellaea*	*truncata*	EF452164.1		EF452110.1	EF452048.1	EU831134.1
Pteridaceae	*Pentagramma*	*pallida*			KR066382.1		
Pteridaceae	*Pentagramma*	*triangularis*	EF452165.1	JX313631.1	EF452111.1	EF452049.1	
Pteridaceae	*Pteris*	*cretica*	EF452170.1	KF289524.1	EF452118.1	EF452055.1	
Pteridaceae	*Pteris*	*tremula*	EF452174.1	KF289520.1	EF452122.1	EF452059.1	AY459164.1
Pteridaceae	*Pteris*	*vittata*	EF473709.1	KF289512.1	EF452123.1	EF452060.1	
Thelypteridaceae	*Thelypteris*	*nevadensis*					AF425178.1
Thelypteridaceae	*Thelypteris*	*puberula*					
Woodsiaceae	*Athyrium*	*distentifolium*	EF463304.1		EF463901.1	EF463560.1	
Woodsiaceae	*Athyrium*	*filix-femina*	FJ821348.1	JF303941.1	FJ821363.1	EF463561.1	AF425152.1
Woodsiaceae	*Cystopteris*	*fragilis*	JX874043.1	JX873985.1	JF832108.1	HQ157273.1	AF425148.1
Woodsiaceae	*Woodsia*	*oregana*	KF186523.1		KF186555.1		
Woodsiaceae	*Woodsia*	*plummerae*	JF832088.1	JF832295.1	JF832149.1	JF832185.1	
Woodsiaceae	*Woodsia*	*scopulina*					