

Limited hydraulic adjustments drive the acclimation response of *Pteridium aquilinum* to variable light

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- **Background and Aims:** The success of invasive plants can be attributed to many traits including the ability to adapt to variable environmental conditions. Whether by adaptation, acclimation or phenotypic plasticity, these plants often increase their resource-use efficiency and, consequently, their fitness. The goal of this study was to examine the hydraulic and eco-physiological attributes of sun and shade populations of *Pteridium aquilinum*, a weedy fern, to determine whether the presence of vessels and other hydraulic attributes affects its success under a variety of light conditions.
- **Methods:** Hydraulic traits such as cavitation resistance, hydraulic conductivity, photosynthesis and water potential at turgor loss point were measured on fronds from sun and shade populations. Anatomical and structural traits such as conduit diameter and length, stomatal density and vein density were also recorded. Diurnal measures of leaf water potential and stomatal conductance complement these data.
- **Key Results:** Gas exchange was nearly double in the sun plants, as was water-use efficiency, leaf-specific conductivity, and stomatal and vein density. This was largely achieved by a decrease in leaf area, coupled with higher xylem content. There was no significant difference in petiole cavitation resistance between the sun and shade leaves, nor in xylem-specific conductivity. Hydraulic conduit diameters were nearly equivalent in the two leaf types.
- **Conclusions:** Shifts in leaf area and xylem content allow *P. aquilinum* to occupy habitats with full sun, and to adjust its physiology accordingly. High rates of photosynthesis explain in part the success of this fern in disturbed habitats, although no change was observed in intrinsic xylem qualities such as cavitation resistance or conduit length. This suggests that *P. aquilinum* is constrained by its fundamental body plan, in contrast to seed plants, which show greater capacity for hydraulic adjustment.

Key words: Plasticity, xylem, embolism, turgor loss point, stomatal density, stomatal conductance, photosynthesis, modulus of elasticity.

INTRODUCTION

The success of invasive or weedy species may be due to a combination of factors, including adaptive physiology. One theory suggests that ‘phenotypic divergence’ confers a competitive edge because invaders may have traits that fall outside of the norm in a given community (Hutchinson, 1959; MacArthur and Levins, 1967; Ordóñez *et al.*, 2010). Alternatively, ‘phenotypic convergence’ may allow an invader to better adapt to local conditions because its traits are more closely aligned with the native plant community (Smith and Knapp, 2001; Curtis, 2003; Ordóñez *et al.*, 2010). Key studies have shown that some invasive species use resources such as light, water and nutrients more efficiently than natives, supporting the former hypothesis (Funk and Vitousek, 2007). Physiological acclimation or phenotypic plasticity may play an important role in all these scenarios allowing invaders to maximize fitness in a novel environment (Davidson *et al.*, 2011). For example, a few co-ordinated changes allow leaves to alter their morphology and physiology to variable levels of light. Sun leaves exhibit higher rates of leaf water transport (K_{LEAF}) and thus higher rates of gas exchange (Boardman, 1977; Bassow and Bazzaz, 1998; Scoffoni *et al.*,

2018), and these functional differences are linked to structural changes such as shifts in vein density and stomatal density, which support increased K_{LEAF} (Brodribb *et al.*, 2007; Murphy *et al.*, 2014; Scoffoni *et al.*, 2015). Hydraulic attributes are rarely examined in studies of invasive species’ physiology, so our goal here was to capture the structural, hydraulic and photosynthetic adjustments of sun and shade populations of bracken fern, *Pteridium aquilinum*, a weedy cosmopolitan fern.

The vast majority of ferns are found in shady understory habitats where evapo-transpirative demand is typically low. With underground rhizomes that give rise to leaves, terrestrial ferns have an ancestral body plan that relies solely on tracheid-based primary xylem, imposing a hard limit on leaf water transport (Brodribb, 2007; Pittermann *et al.*, 2011, 2013, 2015). However, *P. aquilinum* (Dennstaedtiaceae) is one of a limited number of ferns to tolerate full sun, and one of the very few known ferns to have evolved true xylem vessels (Carlquist and Schneider, 2007; Pittermann *et al.*, 2011). Xylem vessels appear later in the fossil record than tracheids and are more morphologically flexible with respect to lumen diameter and conduit length (Aloni, 2015; Wilson, 2016). Hence, the evolution of

vessels has generally led to greater hydraulic efficiency and faster growth rates in angiosperms compared to conifers and ferns, allowing angiosperms to explore a broader morphospace of vascular structure and function (Spicer and Groover, 2010; Aloni, 2015; Gensel, 2018). There is little doubt that hydraulic attributes contributed to the ecological success of angiosperms and so we wondered whether this success may be shared by *P. aquilinum*, one of the most widespread vascular plants that occupies both low light and full sun habitats.

Pteridium aquilinum is a weedy and invasive fern that grows globally throughout temperate and subtropical regions (Page, 1982, 2002). It is a nuisance species, difficult to eradicate, toxic to both humans and livestock if consumed in large quantities, and it often pollutes local water sources with its particular blend of chemical constituents (Marrs and Watt, 2006; Vetter, 2009; Robinson et al., 2010). Ecological disturbances, principally from human activity, have expanded the range of *P. aquilinum*, where it responds to environmental heterogeneity with what appears to be considerable phenotypic plasticity (Page, 1982; Marrs and Watt, 2006; Der et al., 2009). *Pteridium aquilinum* can grow in low-nutrient habitats (Watt, 1940; Cary and Pittermann, 2018) and it can tolerate a range of light levels, from full sunlight to near complete shade (Page, 1976; den Ouden, 2000; Marrs and Watt, 2006). Fronds from either of these sites have a strikingly different appearance. Fronds in full sun are short, with a vertical orientation and thick cuticles (Boodle, 1904; den Ouden, 2000; Fig. 1). By contrast, fronds in the understorey can reach over 1.5 m in length and have a larger leaf area. The ability of *P. aquilinum* to flourish so well in such diverse habitats may due to its adaptive vascular traits (Marrs and Watt, 2006).

Given the functional importance of vessels and the success of *P. aquilinum* across different habitats, we hypothesized that sun-adapted fronds of *P. aquilinum* are likely to have more efficient water transport, as evidenced by higher rates of gas exchange, leaf-specific hydraulic conductivity (K_{LEAF}), xylem specific conductivity (K_s), a tendency toward long,

wide conduits, higher leaf vein and stomatal density, as well as greater resistance to drought-induced embolism in the frond petioles. By contrast, shade-adapted fronds may exhibit lower rates of photosynthesis, reduced phloem content, higher turgor loss point and thinner leaves. We focused primarily on water relations and hydraulic attributes because of their crucial link to plant survival and their value for comparison to vessel-bearing angiosperms.

MATERIAL AND METHODS

Plant material

Fronds of *P. aquilinum* (L.) Kuhn were sampled from wild populations within 1 km of one another on the University of California, Santa Cruz campus reserve during mid-summer (2014–2017). Page (1976) classified the regional subspecies as *Pteridium aquilinum* ssp. *lanuginosum-pubescens*. The campus reserve is located on California's coastal range, which experiences a Mediterranean climate modulated by summer fog (Dawson, 1998). Shaded populations were along road cuts, trails or under moderate canopy cover of redwood (*Sequoia sempervirens*) forest, growing in permeable, black silty loam soils topped with a dense layer of redwood leaf duff (Haff et al., 2008; Fig. 1). The sun populations grew in open south-sloping meadows of invasive grasses on a sandy, light brown loam with a thin cover of organic matter (Haff et al., 2008; Fig. 1). Sample size is six to eight fronds per 'treatment' unless otherwise indicated.

Several attempts at cultivating *P. aquilinum* from rhizome cuttings and spore germination in a common garden setting failed, and this difficulty was confirmed by others in correspondence. Apparently, long segments of the rhizome with a terminal bud must be removed for successful cultivation, but we were not granted permission to disrupt soils on campus. Based on conversations with experts (J. Der, California State University, Fullerton, CA) and the close proximity of the campus populations, we strongly suspect that the sun and shade plants comprise the single regional subspecies. However, in the absence of common garden experiments, we refrain from attributing any phenotypic response to plasticity.

Diurnal measures

Habitat conditions and water relations of sun and shade *P. aquilinum* plants were monitored hourly from 0700 to 1900 h in early June 2015 ($n = 6$ shade plants, $n = 6$ sun plants). These parameters consisted of temperature, wind speed and relative humidity (Kestrel 3000 Wind Meter, Kestrelmeters.com, Minneapolis, MN, USA). Vapour pressure deficit (kPa) was calculated from air temperature and relative humidity. Photosynthetically active radiation (PAR; $\mu\text{mol m}^{-2} \text{s}^{-1}$) was determined with a PAR sensor (LI-250A Light Meter, LI-COR, Lincoln, NB, USA) and volumetric water content (%) was measured in the morning using a soil moisture probe (Hydrosense II, Campbell Scientific, Logan, UT, USA). An SCI-1 porometer was used to measure stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) and leaf temperature (Decagon Devices, Pullman, WA, USA), with

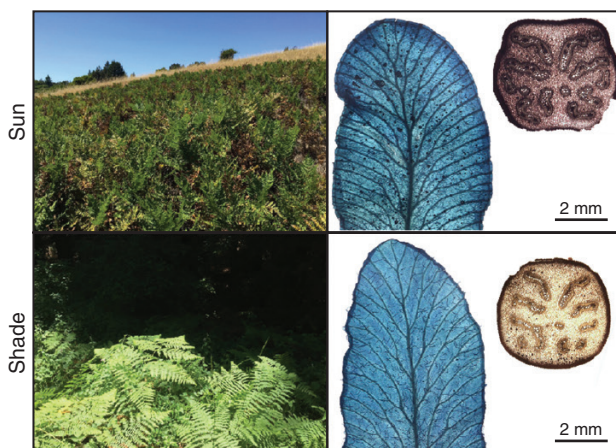


FIG. 1. Examples of sun and shade populations of *P. aquilinum* on the University of California Santa Cruz campus. Sun populations (top panels) are vertically oriented, and have thicker leaves with more pronounced venation than the shade-grown fronds (bottom panels). Sun fronds have a greater petiolar fraction of vascular tissue (top right) than shade plants (bottom left).

stomatal conductance for each individual plant reported as the average of three measures from each frond. Water potential (MPa) was measured on apical pinnae using a Scholander pressure chamber (1505D-EXP, PMS Instrument Co., St. Albany, OR, USA).

Hydraulic conductivity and vulnerability curves

Plants are variably adapted to the drought-induced entry and expansion of air into xylem conduits known as embolism, which may severely disrupt water transport. To examine this in *P. aquilinum*, we measured the general susceptibility of petiole xylem to embolism in fronds from both sun and shade populations, as well as their native levels of embolism.

Vulnerability curves were constructed on eight fronds from each population. On the day of collection, healthy fronds were bagged for 15 min before 0900 h. Fronds were then cut under water at the petiole base and placed in two plastic bags with wet paper towels for transport to the lab. Once there, the leaf petioles were excised and re-trimmed under water, then degassed at -95 kPa to remove any emboli from the xylem conduits. The segments were then fitted to a hydraulic apparatus filled with 20 mM KCl solution to drive flow. Maximum hydraulic conductivity (K_{MAX} , $\text{kg m MPa}^{-1} \text{ s}^{-1}$) was computed as the mass flow rate standardized for segment path length (0.142 m), with a correction for any background flow at 0 kPa taken before and after pressure flow at ~ 6 kPa (Hacke et al., 2000). K_{MAX} was divided by the xylem and leaf area to determine xylem-specific hydraulic conductivity (K_S) and leaf specific hydraulic conductivity (K_{LEAF}), respectively.

Following the K_{MAX} measures, vulnerability curves were constructed by spinning the petioles in a Sorvall RC5C centrifuge at speeds known to induce a range of xylem pressures (Thermo Fisher, Scientific, Waltham, MA, USA). The segments were spun for 5 min at each speed in a custom rotor designed for 0.142 m segments; this was done at incrementally higher speeds until samples were 90 % embolized (Alder et al., 1997). Petiole hydraulic conductivity was measured after each spin (K_{SPIN}). These data were used to construct a vulnerability curve, in which the segment percentage loss of conductivity due to embolism (PLC) is plotted as a function of the xylem pressures attained during the spin (Pockman et al., 1995). PLC was computed as:

$$(1 - K_{SPIN}/K_{MAX}) * 100 \quad (1)$$

A standard function was used to find the water potential at which the petioles reached 12, 50 and 88 PLC (P_{12} , P_{50} , P_{88}) (Pamenter and Van der Willigen, 1998). Less than 1 % of the conduits in the petiole were longer than 0.118 m (see below).

Native PLC values were determined on petioles at midday, and this was done in early September, at the end of the growing season. Eight fronds from each population were covered in a black plastic bag for at least 15 min, after which the water potential was measured on pinnae located at mid-rachis. Fronds were cut under water at the petiole base, transported to the lab and processed for hydraulic measures as described above. Native conductivity was measured first (K_{NATIVE}), then the segment was degassed at -95 kPa and K_{MAX} was measured. PLC was computed as described above.

Frond morphology and anatomy

Total frond leaf area was measured using a leaf area meter (LI-3100, LI-COR). Specific leaf area (SLA) was obtained by first measuring leaf area of a frond sub-sample, drying it at 65 °C for 2 d and computing SLA as leaf area divided by leaf dry mass.

Measures of stomatal and vein density were made on one pinule at mid-rachis for each frond. These samples were cleared in 1 M KOH for 2–4 d, and washed in bleach for 1 min to remove trace pigments. The samples were then stained in toluidine blue and mounted onto slides. The leaf abaxial side was photographed at 20 \times magnification, the images were stitched together with Fiji software (Schindelin et al., 2012) and vein density was measured (vein length divided by lamina area; mm mm^{-2}) using Fiji. To capture stomatal density, the pinule was then photographed at four random locations at 200 \times magnification. At each location the number of stomata was counted in regions with an area of 0.725 mm^2 ; we also measured the length (μm) and width (μm) of 20 randomly selected stoma. Stomatal density is presented as the number of stomata observed per unit area (no. mm^{-2}). These photos were also used for measures of epidermal cell area (μm^2) made on ten cells per individual.

Xylem traits were captured on the petioles used to construct the vulnerability curves by making hand-sections with a razor blade. Sections were stained in phloroglucinol and photographed using a light microscope mounted with a digital camera (instruments cited above). For each section, conduit lumen area, xylem area, phloem area and sectional area were measured using ImageJ. Xylem conduit lumen areas were converted to diameters (D) by treating them as circles: where A is the lumen area. Hydraulic diameters were computed according to Kolb and Sperry (1999) as $D_h = \sqrt{\Sigma D^5 / \Sigma D^4}$. Xylem and phloem areas were divided by total area of the cross-section to obtain the xylem and phloem fractional area (F_x and F_p). Leaf area was divided by xylem area for estimates of Huber values.

Mean conduit lengths were also compared between the sun and shade plants. The stipe and rachis of each frond were trimmed underwater to about 45 cm in length and flushed with purified water for 1 h at 100 kPa. A silicone fluid (Rhodosil RTV-14, Bluestar Silicones, Rock Hill, SC, USA) was mixed with a UV-stain (Uvitex OB, Ciba Specialty Chemicals, Basel, Switzerland), dissolved in 1 % chloroform, and then degassed for 2 h as previously described (Venturas et al., 2016). The basal end of the stipe was shaved with a razor blade, mounted into a closed tubing apparatus and injected with the UV silicone mixture for 24 h under 50 kPa of pressure. During injection, fronds were wrapped in a plastic bag with wet paper towels to prevent desiccation. Fronds were cured at room temperature for 48 h in humidified bags following the injection treatment.

Cured samples were sectioned in a transverse plane with a razor blade at 0, 0.7, 1.4, 2.9, 5.8, 11.8 and 24 cm from the injection point. Micrographs of each section were captured using a fluorescence microscope equipped with digital camera (Zeiss Stereo Discover V.12 with AxioCam HRc digital camera, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). Silicone-filled and non-filled vessels were counted from each section using ImageJ. The proportion of filled to non-filled vessels per section was related to an exponential decay function that was used to calculate mean conduit length (Sperry et al., 2005).

Leaf gas exchange

Leaf CO₂ assimilation rate was measured in response to changing CO₂ concentrations using a portable gas exchange analyser (LI-6400XT, LI-COR; $n = 7$ per treatment). CO₂ levels were set to 400, 0, 400, 600, 800, 1000 and 1200 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, PAR was set to 1600 and 1000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for the sun and shade fronds, respectively, and VPD was kept at 1 kPa. Cuvette temperature varied between the sun and shade site at 21.8 ± 0.4 and 24.1 ± 0.4 °C respectively. CO₂ assimilation curves were analysed using the R software package *plantecophys* (Duursma, 2015; R Core Team, 2019), which fits a curve using the Farquhar–von Caemmerer model to obtain values of maximum carboxylation (V_{CMAX}), maximum electron transport rate responsible for RuP₂ regeneration (J_{MAX}) and the mitochondrial respiration in light (R_p ; Farquhar et al. 1980).

Leaf pressure–volume curves

Pressure–volume curves determined differences in osmotic potential (Ψ_{IMAX} , MPa) and the turgor loss point (Ψ_{TLP} ; MPa) between *P. aquilinum* sun and shade leaves. Healthy fronds from wild populations were cut at mid-stipe and transported back to the laboratory in humidified bags. Frond samples were trimmed to mid-rachis underwater and hydrated for ~2 h. Most samples reached water potentials in the range of values measured between predawn and midday.

Hydrated samples were then further trimmed underwater down to apical pinnae of 10–15 cm length, and these were alternatively weighed on an analytical balance and measured for water potential as they dried. Between measures, samples were placed in sealed bags in darkness for about 15 min to equilibrate water content and slow drying rate. Each sample was measured 10–15 times during dry-down, with at least five measures past the inflection point to capture the linear portion of the pressure–volume curve. After dry-down measures, samples were oven dried at 65 °C for ≥ 72 h and then weighed on an analytical balance to determine the dry mass.

Samples were inspected for signs of over-saturation by plotting the mass of water loss in relation to leaf water potential. Points with an initial steep decline in water potential were removed to avoid the analysis of excessively hydrated leaves (Meinzer et al., 2014). Relative water content (RWC) was computed according to:

$$\text{RWC} = (W_f - W_d) / (W_s - W_d) \quad (2)$$

where W_f is fresh weight (g), W_d is dry weight (g) and W_s is saturated weight (g) of the leaf sample. Saturated leaf weight was taken as the intercept of the initial slope – above the inflection point – where leaf water mass corresponded with a water potential of 0 MPa.

Analyses of turgor loss point and osmotic potential were performed by plotting the relative water deficit (RWD; $1 - \text{RWC}$) and inverse water potential. The last three points beyond the inflection point were fitted with a linear regression, and points were then added towards the inflection point until the goodness of fit was maximized. The water potential at which the linear slope intercepts the inverse water potential axis was the maximum leaf osmotic potential (Ψ_{IMAX}). To find the water

potential at the turgor loss point (Ψ_{TLP}), we found the point that best occupied the transition of the linear portion of the data to the curve, using the linear regression. The slope of turgor pressure in relation to RWC represented the leaf modulus of elasticity (ϵ).

Biomechanics and tissue density

Pteridium aquilinum fronds were tested for differences in biomechanical properties. Measured biomechanical properties were modulus of elasticity (MOE), a metric of stiffness, and modulus of rupture (MOR), a metric of strength. Fronds from each habitat ($n = 16$) were cut at the stipe base and transported to California State University Bakersfield. Stipe samples were trimmed down to 20 cm and the end diameters were measured with digital calipers (Marathon Watch Company Ltd, Richmond Hill, Ontario, Canada). Samples were placed into a material properties testing machine (Instron, Norwood, MA, USA) that performed a four-point bending test. We used the approach of Jacobsen et al. (2005) to calculate MOE and MOR.

The tissue density (mass per unit volume) of stipes was determined on the same samples used to test for MOE and MOR. After performing the four-point bending test, undamaged portions of samples of each frond type were trimmed down to 2 cm in length and saturated with water using vacuum infiltration for >12 h. The displacement method was used to measure sample volume using a four-digit precision balance (Sartorius, Göttingen, Germany). Samples were then dried in an oven at 60 °C for ≥ 72 h and weighed to determine the dry mass. Stipe tissue density was calculated as wet volume divided by dry mass.

Statistical analysis

The data were analysed using a two-sample Student's *t*-test, or, if lacking equal variance, using a Welch's *t*-test (R Core Team, 2019). The data were manually inspected for normal distribution and equal variance in R, following methods outlined by Mangiafico (2016). For each test, $\alpha = 0.05$. For ease of reference, all averages, errors (1 s.d.) and test outputs are reported in Table 1 and ranked in order of significance.

RESULTS

The habitat of the sun- and shade-adapted *P. aquilinum* fronds differed considerably. Whereas the sun plants experienced full sun at midday, the sun flecks in the shade habitat occasionally reached over 1400 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 2). The air vapour pressure deficit (VPD) peaked at 1.45 kPa in the sun but there was considerable overlap between the two sites (Fig. 2). Soils from the sun sites had a volumetric water content of 4.33 ± 0.46 %, compared to the 16.45 ± 2.20 % at shade sites. The air was almost still in the shade but wind speeds varied from 0.4 to 1.7 m s⁻¹ in full sun. Consequently, stomatal conductance was close to double in sun plants than in shade plants, peaking midday at 463 ± 76 and 282 ± 5 $\text{mmol m}^{-2} \text{ s}^{-1}$ respectively (Fig. 2). In line with these differences in habitat and leaf water loss, minimum

TABLE 1. Means, standard deviation (s.d.), and Student's or Welch's t-test outputs comparing sun and shade *P. aquilinum* frond traits. Traits are listed by P-value from smallest to largest.

Trait	Abbreviation	Mean \pm 1 s.d.		<i>t</i>	d.f.	<i>P</i>
		Sun	Shade			
Stipe density (kg m ⁻³)		330.38 \pm 33.21	257.61 \pm 21.66	7.340	30	3.55E-08
Specific leaf area (cm ² g ⁻¹)	SLA	65.32 \pm 5.17	271.54 \pm 39.05	-12.825	10	1.56E-07
Stomatal density (mm ⁻²)	SD	100.97 \pm 19.50	35.86 \pm 4.92	9.158	14	2.75E-07
Maximum electron transport rate (μ mol m ⁻² s ⁻¹)	J_{MAX}	167.49 \pm 23.97	72.17 \pm 10.97	9.566	12	5.77E-07
Maximum carboxylation rate (μ mol m ⁻² s ⁻¹)	V_{CMAX}	82.40 \pm 12.14	39.25 \pm 5.49	8.568	12	1.85E-06
Minimum water potential (MPa)	Ψ_{MIN}	-1.83 \pm 0.22	-0.74 \pm 0.29	-7.48	10	2.11E-05
Vein density (mm mm ⁻²)	VD	2.93 \pm 0.35	2.15 \pm 0.14	5.885	14	3.97E-05
Xylem fraction (%)	F_X	8.81 \pm 0.83	6.20 \pm 0.56	6.385	10	7.99E-05
Phloem fraction (%)	F_P	17.15 \pm 0.75	13.89 \pm 1.25	5.483	10	0.0003
Leaf epidermal cell area (μ m ²)		3660 \pm 888	6695 \pm 1077	-5.325	10	0.0003
Maximum stomatal conductance (mmol m ⁻² s ⁻¹)	g_{SMAX}	462 \pm 76	282 \pm 36	5.229	10	0.0003
Leaf to xylem area ratio (m ² cm ⁻²)	$A_L : A_X$	3.94 \pm 2.05	17.82 \pm 5.21	-6.072	6.51	0.0007
Conduit diameter (μ m)	D	24.65 \pm 0.78	21.67 \pm 0.54	6.280	6	0.0008
Leaf specific hydraulic conductivity 10 ⁵ \times (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	K_{LEAF}	26.78 \pm 18.95	5.39 \pm 3.49	5.206	7.92	0.0008
Stipe modulus of elasticity (N mm ⁻²)	MOE	6821 \pm 2009	9091 \pm 1658	-3.484	30	0.0015
Water potential at 88 % loss of hydraulic conductivity (MPa)	P_{88}	-2.59 \pm 0.34	-2.06 \pm 0.20	-3.741	14	0.0021
Water potential at turgor loss point in pinnae (MPa)	Ψ_{TLP}	-2.20 \pm 0.16	-1.72 \pm 0.21	-3.801	8	0.0052
Intrinsic water use efficiency (μ mol CO ₂ mol H ₂ O ⁻¹)	WUE	61.67 \pm 6.59	34.13 \pm 38.94	3.208	12	0.0075
Maximum osmotic water potential in pinnae (MPa)	Ψ_{IMAX}	-1.83 \pm 0.21	-1.48 \pm 0.12	-3.068	8	0.0154
Pinnae modulus of elasticity (MPa)	ϵ	18.84 \pm 3.62	14.44 \pm 3.79	1.965	8.78	0.0818
Water potential at 50 % loss of hydraulic conductivity (MPa)	P_{50}	-1.76 \pm 0.26	-1.51 \pm 0.28	-1.857	14	0.0845
Stipe modulus of rupture (N mm ⁻²)	MOR	80.29 \pm 14.31	73.13 \pm 13.37	1.464	30	0.1536
Percentage loss of hydraulic conductivity at midday (%)	PLC _{midday}	44.22 \pm 25.30	28.18 \pm 20.05	1.4214	18	0.1723
Guard cell length (μ m)		48.58 \pm 2.33	49.95 \pm 1.87	-1.300	13.36	0.2160
Proportion of xylem conduits filled at 0.058 m from silicone injection point (%)		2.38 \pm 0.77	3.31 \pm 1.59	-1.292	10	0.2256
Hydraulic conduit diameter (μ m)	D_H	68.68 \pm 2.68	70.38 \pm 1.38	-1.123	6	0.3044
Guard cell width (μ m)		14.66 \pm 0.63	14.08 \pm 1.20	0.785	14	0.4450
Xylem-specific hydraulic conductivity (kg m ⁻¹ kPa ⁻¹ s ⁻¹)	K_S	21.01 \pm 6.57	23.35 \pm 5.07	-0.690	10	0.5059
Day respiration (μ mol m ⁻² s ⁻¹)	R_D	1.22 \pm 0.23	1.38 \pm 1.09	-0.375	12	0.7141
Mean conduit length (m)	L_C	0.014 \pm 0.004	0.015 \pm 0.005	-0.308	10	0.7645
Proportion of xylem conduits filled at 0.118 m from silicone injection point (%)		0.52 \pm 0.25	0.58 \pm 0.61	-0.180	10	0.8604
Water potential at 12 % loss of hydraulic conductivity (MPa)	P_{12}	-0.89 \pm 0.55	-0.93 \pm 0.59	0.148	14	0.8843

midday frond water potential (Ψ_{MIN}) was consistently lower in the sun populations than in the shade: sun populations reached their nadir at -1.9 MPa, while the shade plants rarely dipped below -0.5 MPa (Fig. 2).

The fronds were also morphologically distinct. Sun fronds were shorter with an average length of 0.68 \pm 0.021 m compared to shade fronds (0.96 \pm 0.05 m), they had 30 % lower leaf area (898 \pm 149 cm²) than shade fronds (1259 \pm 205 cm²) and thicker, tougher leaves as manifest by their significantly higher specific leaf area (SLA; Table 1). Importantly, the sun fronds had more xylem and phloem on a petiole cross-sectional basis (the xylem and phloem fraction respectively, F_X and F_P ; Fig. 3), and when combined with their smaller leaves, their ratio of leaf area to xylem area was 78 % lower than in shade-grown plants ($A_L : A_X$; Fig. 3). Taken together, the sun ferns cope with the greater heat load by reducing leaf area and investing into more substantial vascular infrastructure in comparison with the shade plants.

Both the low-light and the high-light plants showed a surprisingly similar hydraulic response. The specific xylem conductivity of the petiole (K_S) was no different in the sun and

shade plants, although K_{LEAF} was significantly higher in the sun-exposed fronds (Fig. 3). This is because the sun plants have smaller leaves and thus a more favourable leaf area to petiole xylem area ratio (Fig. 3). The similarity in K_S in the two treatments can probably be explained by the fronds' similar hydraulic conduit diameters (D_H), which averaged 70.38 \pm 1.38 and 68.68 \pm 2.68 μ m for the sun and shade leaves respectively (Fig. 3). D_H better reflects xylem hydraulic capacity than mean conduit diameters (D_{MEAN}) because conductance is highly sensitive to even small changes in conduit diameter (Kolb and Sperry, 1999). Nevertheless, at an average diameter of 24.65 \pm 0.78 μ m, D_{MEAN} was significantly higher in the sun than in the shade plants but the absolute difference between the two groups was only 3 μ m (Fig. 3). Mean tracheid/vessel length was equivalent for the two leaf types (1.4 and 1.5 cm for sun and shade leaves, respectively) with less than 1 % of the frond conduits reaching lengths over 11.8 cm (Fig. 3).

Stomatal density (SD) and vein density (VD) were significantly higher in sun leaves than in fronds from the understorey. SD averaged 100.97 \pm 19.5 stomata mm⁻² in the sun leaves, whereas only 35.86 \pm 4.92 stomata mm⁻² were counted in the

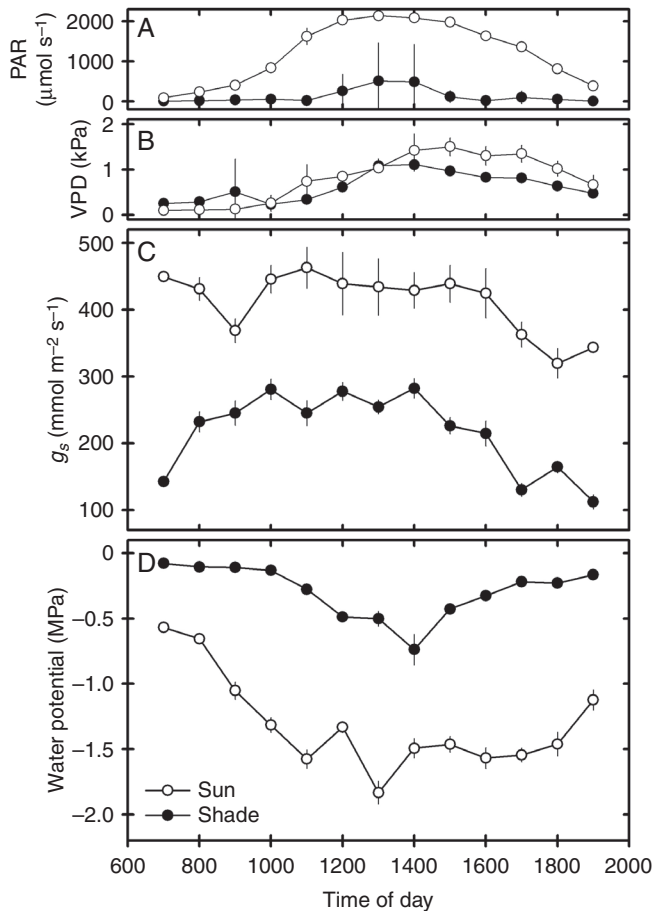


FIG. 2. Diurnal measures of (A) photosynthetically active radiation (PAR), (B) vapour pressure deficit (VPD), (C) stomatal conductance (g_s) and (D) leaf water potential (ψ) in sun and shade populations of *P. aquilinum* (open and closed circles, respectively).

shade leaves, a result that is consistent with the g_{SMAX} of these plants *in situ* (Figs 2 and 3). The response of leaf vein density was functionally co-ordinated with stomatal density; the sun leaves had a VD of $2.93 \pm 0.35 \text{ mm mm}^{-2}$ whereas the VD of shade leaves averaged $2.15 \pm 0.14 \text{ mm mm}^{-2}$ (Fig. 3). Stomatal size, as indicated by guard cell length and width, was no different despite the pronounced difference in stomatal density, although epidermal cells were 45 % smaller in the sun leaves than in the shade leaves (Table 1).

Petiole xylem vulnerability to air-induced embolism was equivalent in the sun and shade plants (Fig. 4). P_{50} ranged from -1.13 to -2.31 MPa across all samples, which is consistent with the water potentials these plants experienced in the field (Fig. 2). All petioles were 100 % embolized between -3 and -4 MPa . Midday petiole PLC values were statistically indistinguishable between fronds from both habitats, but they generally overlapped with their respective vulnerability curves (Fig. 4).

Both the water potential at turgor loss point (Ψ_{TLP}) and the maximum osmotic potential (Ψ_{π}) at full hydration were significantly lower in the sun plants than in those from the shade (Fig. 5), indicating greater resilience to wilting as well as a greater role for osmotic regulation of water potential in

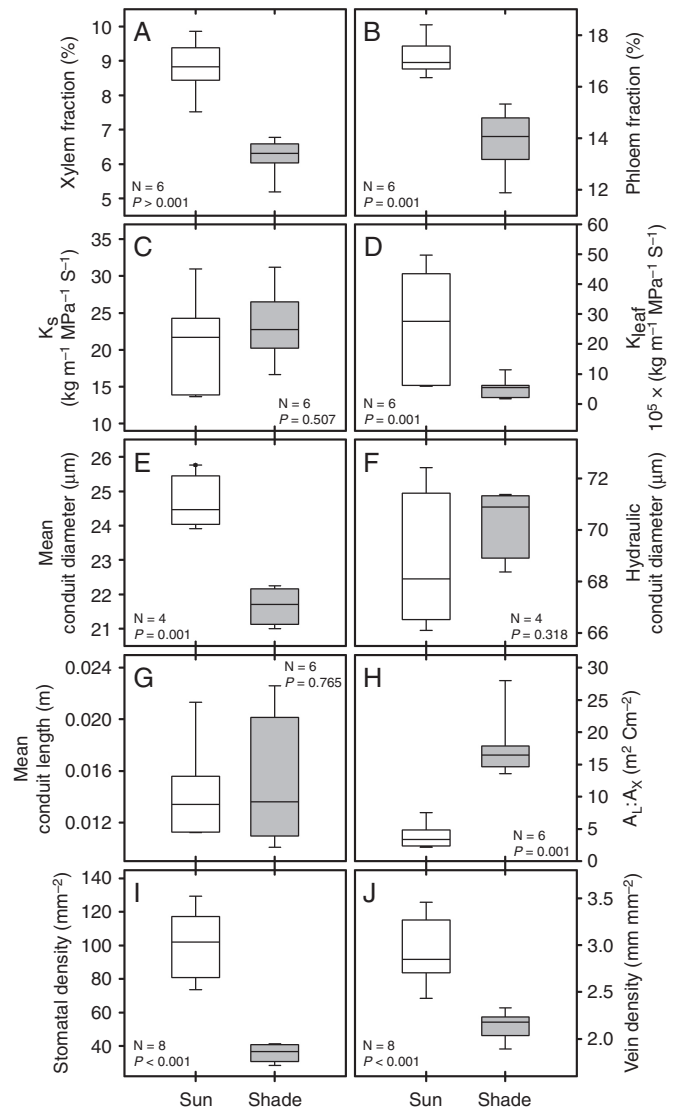


FIG. 3. Hydraulic and anatomical trait comparisons in sun and shade fronds of *P. aquilinum* (white and grey boxplots, respectively): (A, B) cross-petiole xylem and phloem fraction, (C, D) xylem-specific (K_s) and leaf-specific conductivity (K_{LEAF}), (E, F) mean conduit diameter (D) and hydraulic conduit diameter (D_H), (G) mean conduit length (L_c), (H) the ratio of leaf area to xylem area ($A_L:A_X$), and (I, J) stomatal density (SD) and vein density (VD).

sun-exposed plants. The leaf modulus of elasticity was similar in both leaf types (Table 1). Representative pressure–volume curves are available in the Supplementary Data, Information S1.

Consistent with adaptation to higher light levels, the sun-exposed fronds had higher rates of photosynthesis than the shade leaves, peaking at $32 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at a leaf CO_2 (C_i) of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. By contrast, photosynthesis in the shade leaves saturated at a C_i of approximately $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ reached a maximum assimilation rate of $14 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at the maximum C_i (Fig. 6). Sun fronds had more than double the rates of maximum electron transport (J_{MAX}) and carboxylation (C_{MAX}) than the shade leaves, indicating an up-regulation of both the Calvin cycle and light reactions of photosynthesis (Fig. 6). Similarly, intrinsic water use efficiency (WUE) in sun

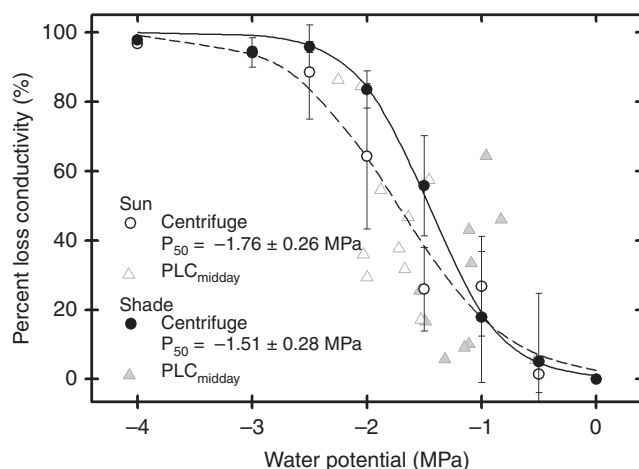


FIG. 4. Percentage loss of petiole conductivity in response to xylem pressure in sun and shade fronds of *P. aquilinum*.

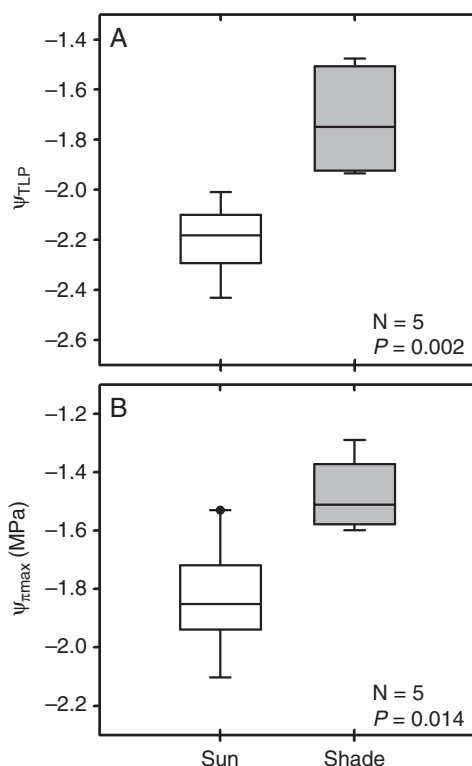


FIG. 5. (A) Water potential at turgor loss point (Ψ_{TLP}) and (B) maximum osmotic potential (Ψ_{IMAX}) in sun and shade fronds of *P. aquilinum*.

plants was almost double that of the shaded fronds (Fig. 6). No differences were observed in dark respiration rates in both plant types (Table 1).

The biomechanical properties of the petioles differed between the sun and shade plants. The sun plants had significantly more flexible petioles, with a modulus of elasticity (MOE) of $6821 \pm 2009 \text{ N mm}^{-2}$ as compared to shade-grown fronds, in which MOE averaged $9091 \pm 1659 \text{ N mm}^{-2}$ (Table 1). While the modulus of rupture was no different in the two treatments,

the sun-grown leaves had significantly denser petioles than the shade plants (Table 1), probably due to the high number of starch grains in the parenchyma tissue (Supplementary Data, Information S2).

DISCUSSION

Vascular adjustment contributed to the success of *P. aquilinum* in its range of habitats but the means by which this was achieved differed from our predictions. Given the evapotranspirative demand encountered by *P. aquilinum* at high light we expected these plants to have a greater fraction of long and wide conduits, as well as higher resistance to cavitation but instead our results show that the intrinsic xylem transport (K_S) and embolism resistance of both sun and shade fronds were, for the most part, equivalent. Large differences in mean conduit diameter were swamped by the contributions of the larger, hydraulically significant conduits (D_H) and an abundance of long vessels in both sun and shade fronds. Similarly, the nearly equivalent P_{50} values in petioles from both populations suggest that neither pit membrane structure nor vascular integration adjusted to the contrasting demand for water as might be expected based on earlier reports (Schoonmaker *et al.*, 2010; Brodersen *et al.*, 2012, 2014). Instead, we observed that the sun leaves had more xylem and phloem on a cross-sectional basis, and that when coupled with a lower leaf area, this led to a near doubling of g_{SMAX} and WUE. Taken together, vascular acclimation of *P. aquilinum* to light is accomplished primarily by reductions in leaf area and a concurrent increase in xylem content.

Smaller, thicker, vertically oriented leaves are characteristic of high light environments (Niinemets, 2010; Leigh *et al.*, 2012; Sessa and Givnish, 2014; Scoffoni *et al.*, 2015), and this was also a key attribute of sun-adapted *P. aquilinum* fronds. Reduced leaf area promotes water conservation while a vertical disposition reduces photoinhibition and heat loading (Givnish, 1988). Thicker leaves have the additional advantage of buffering thermal fluctuations caused by variation in light intensity and air speed (Leigh *et al.*, 2012), which too is adaptive for the sun populations of *P. aquilinum*. Indeed, *P. aquilinum* sun leaves had greater specific leaf area than those growing in the shade probably due to increased lignification, as observed in *P. aquilinum* elsewhere (Jones, 1983). Exposed fronds in high sun environments are subject to persistent agitation by wind, so along with tougher leaves, the petioles of the sun fronds were more flexible, as evidenced by their lower MOE compared to the shade plants (Table 1). A concurrent explanation is that the petioles of the shade plants were stiffer to support their higher leaf areas.

The high rates of photosynthesis in *P. aquilinum* are exceptional among ferns. Most ferns occupy low-light environments such as the forest understorey, so their assimilation rates rarely exceed $10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Brodribb *et al.*, 2007; Watkins *et al.*, 2010; Pittermann *et al.*, 2011; Sessa and Givnish, 2014). However, the sun plants of *P. aquilinum* had leaf to xylem area ratios that were over four times lower than those of the understorey populations, thereby significantly increasing their K_{LEAF} (Figure 3; Table 1). Leaf-specific conductance must be functionally co-ordinated with appropriate stomatal and vein densities, both of which were observed to be greater in the sun

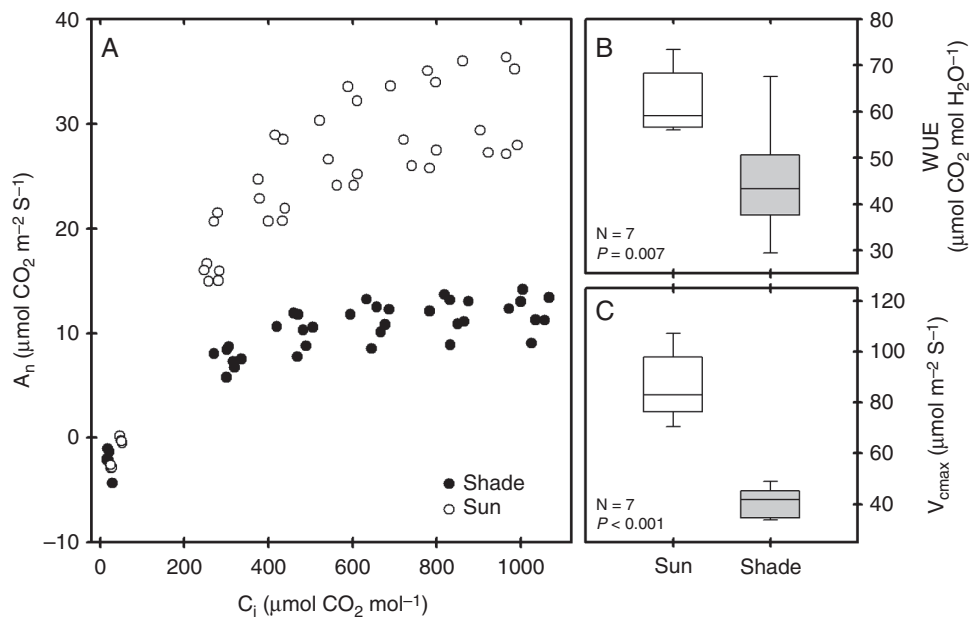


FIG. 6. Gas-exchange response of sun and shade fronds of *P. aquilinum* (open and shaded boxplots, respectively). (A) The response of assimilation (A_n) to increasing leaf CO_2 levels (C_i). (B, C) The intrinsic leaf water-use efficiency (WUE) and maximum carboxylation rate (V_{cmax}).

fronds than in shade-grown plants (Scoffoni *et al.*, 2015). That being said, we suspect that it was the reduction in leaf area that led to increased stomatal and vein density in sun leaves, rather than developmental up-regulation in these traits *per se* because the epidermal cells shrank in size while stomatal size remained the same (Table 1). The consequence of this hydraulic and anatomical co-ordination was that the sun plants had much higher rates of gas exchange than the low-light population (Figs 3 and 6; Table 1; see also Brodribb *et al.*, 2007; Sessa and Givnish, 2014; Scoffoni *et al.*, 2015). Previous studies of *P. aquilinum* physiology report assimilation values up to $15 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at a C_i of $400 \mu\text{mol mol}^{-1}$ (Caporn *et al.*, 1999) but several sun leaves from the Santa Cruz sun populations achieved rates beyond $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 6). High rates of carbon uptake may allow *P. aquilinum* to grow quickly, and thus spread and out-compete existing vegetation, especially following frost, fire or other disturbance (Marrs and Watt, 2006).

Pteridium aquilinum plants from both sun and understory populations operate perilously close to wilting. The ψ_{MIN} of the sun plants reached -1.83 ± 0.22 MPa, only 0.37 MPa away from the water potential at turgor loss point, and practically equivalent to their average P_{50} (Table 1). By contrast, the safety margins are slightly higher in the shade-grown fronds at 0.74 MPa from the turgor loss point and 0.77 MPa from the P_{50} . Studies on *Dryopteris arguta*, a perennial, similarly sized fern that is widely distributed on North America's west coast, revealed that southern California populations are capable of significant osmotic adjustment, reaching an osmotic potential at turgor loss point below -6 MPa at the end of the dry season (Holmlund *et al.*, 2016). *Pteridium aquilinum* is drought-deciduous and thus unlikely to adjust to the same extent. The native PLC values are consistent with the vulnerability curves, as well as the expectation that shade-growing individuals should experience milder conditions and thus less embolism. It is also possible that these

ferns experience some degree of transport failure during the growing season, but given their high hydraulic capacity and redundancy (Brodersen *et al.*, 2012), this may not significantly impact leaf function.

Pteridium aquilinum is a noxious, cosmopolitan and highly adaptive weed. Large fronds may have xylem with vessels in excess of 15 cm in length, its conduit diameters are on par with those of some ring-porous species, and its vein and stomatal densities are on the high end of the range relative to other ferns (Boyce *et al.*, 2009; Franks and Beerling, 2009; Pittermann *et al.*, 2011). Yet despite its physiological capacity, this outlier fern remains, to a large extent, hydraulically canalized in that its acclimation to variable light levels consisted of simple adjustments in leaf area, epidermal cell size and xylem content. In contrast to angiosperms and conifers which may alter conduit size and pit membrane attributes (Plavcová and Hacke, 2012; Bryukhanova and Fonti, 2013; Petit *et al.*, 2016), *P. aquilinum*'s vulnerability to cavitation remained largely unchanged, as did the fundamental attributes of its xylem tissue. Neither did the presence of vessels confer any particular hydraulic advantage to the sun-exposed fronds. *Pteridium aquilinum*'s apparent adjustments are functionally adept but they are also relatively straightforward given that they entail the addition of existing tissue types rather than their fine-tuning. This may be part of a broader suite of limitations among ferns that may be related in part to their ancestral physiology and the absence of a vascular cambium (Pittermann *et al.*, 2011; Brodribb *et al.*, 2017). Alternatively, this response could, quite simply, be specific to our target taxon. Four species comprise the genus *Pteridium*, with *P. aquilinum*, *P. caudatum*, *P. esculentum*, *P. semihastatum* and their numerous subspecies located around the world (Marrs and Watt, 2006). Further research into *Pteridium*'s adaptive physiology may reveal other functional strategies that confer this genus with such exceptional competitive ability.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following.

Information S1: Examples of pressure–volume curves generated from sun and shade fronds of *P. aquilinum*.

Information S2: The parenchyma tissue from petioles in sun fronds of *P. aquilinum* were packed with starch grains, relative to the shade fronds.

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