Photoprotection of PSII in Hawaiian lobeliads from diverse light environments

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Abstract. Excess irradiance can reduce the quantum yield of photosynthesis via photoprotective energy dissipation, inactivation or downregulation of PSII. We examined variation in photoprotection as part of a study of adaptive radiation in photosynthetic light responses by Hawaiian lobeliads. We measured the maximum efficiency of PSII (F_v/F_m) and recovery of F_v/F_m after high light stress in field populations of 11 lobeliad species and in four species growing under common-garden greenhouse conditions. Species showed no difference in F_v/F_m (0.82 ± 0.02 (mean $\pm$ s.e.)) or in their ability to recover from light stress under field conditions. Average recovery was $74 \pm 1.4\%$ within 1 h of removal of the stress suggesting that all species maintain the ability to recover from high light stress, at least in the short-term. In contrast, the results from the common-garden indicate that long-term exposure to high irradiance and associated higher temperatures can cause a sustained reduction in PSII function. Species showed decreased F_v/F_m and percentage recovery as treatment irradiance increased. F_v/F_m and percentage recovery were positively related to native habitat PFD across species, suggesting that there has been a diversification in high light tolerance, with species from sunnier environments better able to avoid sustained declines in PSII function.

Additional keywords: adaptive radiation, chlorophyll fluorescence, common garden, non-photochemical quenching, photochemical reflectance index, xanthophyll-cycle pigments.

Introduction

Extreme isolation, a long geological history and extensive ecological variation make the Hawaiian islands a hotspot for adaptive radiation (Robichaux *et al*. 1990; Kambysellis and Craddock 1997; Sakai *et al*. 1997; Jordan *et al*. 2003; Gillespie 2004). Of these radiations, the Hawaiian lobeliads (Campanulaceae, six endemic genera, 125 species) represent one of the most striking (Rock 1919; Carlquist 1965, 1970; Lammers 1990; Givnish *et al*. 1995; Givnish 1998). The radiation of lobeliads includes alpine rosette shrubs, bizarre cliff succulents, and shrubs, trees and vines of tropical forest edges and interiors. Indeed, since their colonisation ~13 Ma, the lobeliads have invaded a broad range of light habitats (ranging from 2.3 to 30.0 mol photons m^{-2} day^{-1}) on predominantly moist substrates and have become the most diverse plant group on any single archipelago derived from a single colonisation event (Givnish *et al*. 2004). Thus, the Hawaiian lobeliads provide a model system for studying adaptive radiation of photosynthetic adaptations to different light regimes. Previously, we have shown diversification in maximum photosynthetic rates, dark respiration rates, sunfleck use efficiency, the rapidity of photosynthetic induction, leaf N content, specific leaf area (SLA), and other traits associated with leaf photosynthetic physiology (Givnish *et al*. 2004; Montgomery and Givnish 2008). The trends observed include increases in photosynthetic

rates, respiration rates and leaf N, decreasing sunfleck use efficiency and slower induction of photosynthesis as habitat light availability increases. In the present study, using both field and common-garden approaches, we examined whether the lobeliads vary in their ability to cope with the stresses associated with excess light.

Although commonly viewed as beneficial, high light places considerable stress on the photosynthetic apparatus. In plants that have been exposed to high light, the efficiency of photosynthesis decreases because of an increase in the number of photons absorbed per CO_2 molecule assimilated at low light levels. This decline in the quantum yield of PSII can cause both short- and long-term reductions in photosynthesis depending on the extent of the photoprotective, recovery and repair processes (Krause 1988; Cavender-Bares and Bazzaz 2004). Field studies have shown that unstressed plants show remarkably constant potential quantum yields of PSII, as measured by a chlorophyll fluorescence ratio (F_v/F_m) of ~0.80 (Björkman and Demmig 1987), and chlorophyll fluorescence has emerged as a sensitive indicator of various environmental stresses, including light stress (Cavender-Bares and Bazzaz 2004). Among species that differ in shade tolerance, species restricted to high light habitats show a higher capacity to tolerate and recover from light stress than more shade-tolerant species (Kamaluddin and Grace 1992; Johnson 1993;

Brugnoli *et al.* 1994; Lovelock *et al.* 1994; Demmig-Adams *et al.* 1995; Houter and Pons 2005). Within species, leaves developed under sunnier conditions show a more rapid recovery of F_v/F_m after exposure to high light stress at the scazle of long sunflecks and gap formation (Kamaluddin and Grace 1992; Mulkey and Pearcy 1992; Brugnoli *et al.* 1994; Valladares and Pearcy 1997; Demmig-Adams *et al.* 1998; Lovelock *et al.* 1998). In shaded habitats, sunflecks can lead to short-term reductions in the quantum yield of photosynthesis as a result of low photosynthetic induction and the generally low photosynthetic capacity of shade-adapted plants (Watling *et al.* 1997). Interestingly, in a suite of tropical understory plants, Lovelock *et al.* (1998) found that leaf lifespan positively correlates with recovery from short-term, high light stress.

Photoprotective mechanisms are critical for individuals growing in sun-exposed sites and for shaded individuals during intense sunflecks. Depending on the species and the habitat of origin, such plants use only 10–50% of peak irradiance for photosynthesis (Demmig-Adams and Adams 1996*b*). Although some plants avoid absorbing excess radiation through leaf movements, chloroplast re-orientation and increased reflectance, most plants rely strongly on biochemical means to dissipate excess energy and to avoid damage to the photosystems (Bilger and Björkman 1990; Johnson 1993; Fetene *et al.* 1997; Demmig-Adams 1998). One of the principal means of photoprotection occurs through xanthophyll-cycle-dependent energy dissipation in high light.

Both the biochemistry of the xanthophyll cycle and its function and variation in ecological systems have been extensively studied (Adams and Demmig-Adams 1993; Gilmore and Yamamoto 1993; Johnson 1993; Demmig-Adams and Adams 1996*b*). Pool sizes of xanthophyll-cycle pigments in the thylakoid are positively correlated with the amount of excess energy dissipated (Demmig-Adams and Adams 1996*a*) and increase as growth light increases (Demmig-Adams 1998; Logan *et al.* 1998*a*, 1998*b*). Xanthophyll-cycle pool sizes and energy dissipation are also sensitive to sunfleck activity (Logan *et al.* 1997; Demmig-Adams 1998). For example, Logan *et al.* (1997) found that individuals of *Alocasia brisbanensis* (Bailey) Domin in deep shade had significantly lower levels of xanthophylls compared with other understory individuals from microhabitats with high sunfleck activity.

Despite the potential negative effects of excess light, considerable inactivation of PSII can occur under high light conditions without a significant effect on carbon assimilation: under high light conditions the energy absorbed exceeds the downstream capacity to use that energy (Lee *et al.* 1999; Kornyeyev *et al.* 2006). Declines in the efficiency of PSII as a result of photoinactivation and/or downregulation of PSII might reduce carbon gain under conditions where electron transport limits carbon gain (e.g. shady conditions); however, rates of carbon export can also play a role in limiting photosynthetic acclimation to changing light conditions (Adams *et al.* 2007). As photoprotective dissipation of excess energy is an important component of acclimation and adaptation to the light environment, we expect diversification in traits related to photoprotection in lineages, like the Hawaiian lobeliads, that span strong light gradients.

In the present study, we examined how the Hawaiian lobeliads have diversified in their response to high light stress. Although we expected all field-grown plants to show similar F_v/F_m as a consequence of acclimation and adaptation to their environments, we hypothesised that species from high light habitats would show more rapid and extensive recovery after high light stress. Under common-garden greenhouse conditions, we hypothesised that species from high light habitats would suffer reduced long-term effects of high light stress (manifested by higher F_v/F_m) and would show more rapid recovery after stress. Furthermore, we examined the mechanisms to reduce and dissipate levels of excess light, hypothesising that species from high light environments would possess greater photochemical and non-photochemical mechanisms to dissipate light energy. Our common-garden approach (four light levels) allowed us to examine whether the responses of species to different light environments primarily resulted from their growth environment or from inherent differences among species.

Materials and methods

Study sites and species

We studied 11 species representing each major clade of Hawaiian lobeliads (Table 1). We chose species native to a narrow elevational range (1000–1250 m a.s.l.) in areas with moderate to heavy rainfall (>1500 mm year^{-1}). *Brighamia* does not meet these criteria, but is adapted to cooler, effectively moister conditions than expected based on elevation, given the strong onshore breezes and trade winds to which it is exposed in its natural sea-cliff habitat (Table 1). Detailed data on the study populations are given by Givnish *et al.* (2004). Whenever possible, we measured individuals of each species in one to three different populations growing under relatively undisturbed natural conditions. It proved impossible to access the rare natural populations of two federally endangered species (*Brighamia* and *Delissea*). These species were studied at outplantings established by the National Tropical Botanic Garden at Limahuli. Individuals included from outplantings had been grown from seeds collected from the wild.

Cultivation methods and environment

Our common-garden greenhouse was located at the Hawai'i Agricultural Experiment Station of the University of Hawai'i in Volcano, HI, in the heart of the elevational range of the lobeliad lineage (1190 m). The greenhouse was a hoop house with a plastic cover, open to ambient air along the lower walls (0–3.5 feet off the ground) and at both ends of the structure. The greenhouse was divided into four light treatments that provided 6, 12, 35 and 80% of full sun. Light levels were achieved using a combination of neutral density shadecloth and plastic film that adjusted the red : far red (R : FR) ratio. We matched R : FR ratios in our four greenhouse treatments to R : FR levels measured in the field. The R : FR ratios were assessed using a field portable spectroradiometer (Uni-Spec; PP-Systems, Amesbury, MA, USA). Field measures of R : FR were made at the beginning of the study. The R : FR ratios were as follows: 0.53, 6% full sun treatment; 0.62, 12% treatment; 0.72, 35% treatment; and 0.89,

Table 1. Daily sunfleck activity experienced by field populations of 11 species of Hawaiian lobeliads

Superscript letters indicate means that differ significantly from each other ($P < 0.05$). Species are ordered from lowest to highest daily photon flux density (PFD)

Species	Daily PFD (mol m^{-2} day^{-1}) Mean	s.e.	Sensors (N)	Sunfleck length (min) Mean	s.e.	Sunfleck length (min) Maximum	s.e.	Daily minutes of sunflecks Mean	s.e.	Sensors (N)
Cyanea floribunda	2.3[a]	0.2	49	1.4[e]	1.7	21.4[g]	19.6	31[f]	21	48
Cyanea pilosa	3.2[a]	0.4	52	1.5[e]	2.3	30.3[fg]	27.2	67[ef]	29	25
Cyanea hirtella	6.2[ab]	0.9	11	2.9[de]	3.5	117.5[efg]	41.0	191[cde]	43	11
Cyanea leptostegia	7.0[b]	0.6	29	6.0[de]	2.2	140.6[ef]	25.3	195[d]	27	29
Clermontia parviflora	8.3[b]	0.8	52	8.8[cde]	1.6	215.7[de]	18.2	243[d]	19	56
Lobelia yuccoides	15.1[c]	2.4	25	1.9[e]	2.1	28.3[fg]	24.4	384[b]	26	31
Delissea rhytidosperma	15.3[c]	2.1	13	19.7[abc]	3.7	392.5[bc]	60.5	384	51	14
Clermontia fauriei	15.8[c]	1.3	59	13.2[bcd]	1.9	291.5[cd]	21.5	340[bc]	23	40
Trematolobelia kauaiensis	21.1[d]	2.1	31	31.2[a]	2.1	505.3[ab]	24.8	517[a]	26	30
Lobelia villosa	28.4[e]	1.1	23	25.0[abc]	4.8	640.6[a]	55.5	533[ab]	59	6
Brighamia insignis	30.0[e]	3.0	12	24.9[ab]	3.4	220.3[de]	39.3	226[cde]	42	12

80% treatment. The average daily temperature ranged from 16.5°C in the 6% light treatment to 18.2°C in the 80% light treatment. The greenhouse was equipped with an automatic misting system to deliver water on a regular basis and to maintain high humidity levels. Plants were also hand watered as necessary to maintain a non-limiting water supply. Plants received slow release fertiliser (Osmocote, 14-14-14; The Scotts Miracle-Gro Company, Marysville, OH, USA) in the potting media and weekly foliar fertiliser treatments (Gaviota 60, 19-19-19; J. R. Simplot, Boise, ID, USA). The plants were initially started on a vermiculite/perlite media in trays covered with clear grow domes and transferred to individual pots when they were ~5 cm tall. For the remainder of the experiment the plants were grown in cinder soil and transplanted regularly as they grew to minimise pot effects. At the end of the present study, plants were growing in 3–5 gallon pots and ranged in height from 0.5 to 1.25 m. The plants were ~1.5 years old. Our care and cultivation methods were based on the extensive experience in propagation of rare lobeliads at the adjacent Hawaiian Endangered Plant Facility.

Light availability

We measured the daily courses of photon flux density (PFD; μmol photons m^{-2} s^{-1}) over 5–12 individuals in each study population using GaAs photodiodes (Hammamatsu, Bridgewater, NJ, USA) connected to dataloggers (Campbell Scientific, Logan, UT, USA). The PFD data were logged at 10-s intervals. For a detailed description of the calibration of the sensors and of the measurements see Givnish *et al.* (2004). We calculated the number of minutes of sunflecks, the average duration of the sunflecks and the maximum sunfleck length for each species. Sunflecks were defined as periods of light above 150 μmol photons m^{-2} s^{-1}.

Chlorophyll fluorescence

We assessed *in situ* chlorophyll fluorescence with a PAM fluorometer (Mini-PAM, Walz, Germany). We measured dark-acclimated F_v/F_m as an indicator of photosynthetic function and stress. F_v/F_m is the ratio of variable ($F_m - F_o$) to maximal fluorescence (F_m), where F_m is the maximal fluorescence level when all PSII reaction centers are closed and F_o is the minimal fluorescence level when all PSII reaction centers are open. F_v is the maximum variable fluorescence when non-photochemical processes are at a minimum.

We chose the newest fully expanded leaf on five individuals of each species. To obtain F_v/F_m, the leaves of field plants were covered in the early morning with heavy black cloth and an umbrella was also used to shade the plant and reduce the possibility of heating of the leaf. While pre-dawn measures of F_v/F_m are preferable (Logan *et al.* 2007), the remote locations of the field populations made pre-dawn measurement difficult. We considered shading the leaves with black cloth the day before measurement, but the constant possibility of torrential rainfall at our field sites made this approach untenable. Greenhouse plants were kept in a darkened laboratory before measurement. All measurements were started after the leaves had been darkened for at least 60 min. We felt that this was sufficient time for oxidation of the electron transport chain and disengagement of any thermal energy dissipation because early morning shading meant that none of our plants had experienced levels of PFD sufficient to saturate photosynthesis (e.g. at most ~1 h of PFD <150 μmol m^{-2} s^{-1}).

After assessing F_v/F_m, the plants were subjected to 20 min of high light (2500 μmol photons m^{-2} s^{-1} delivered by an external light source) followed by a 40-min recovery period in the dark. During the high light and recovery periods we measured the fluorescence at 1-min intervals and estimated the actual efficiency of PSII in the light (from $[F_m' - F]/F_m'$), non-photochemical quenching (NPQ) of F_m' and F_v/F_m. This protocol had been used in a previous study that compared native and invasive ferns and was sufficient to cause impaired recovery in the native fern (Durand and Goldstein 2001). The PFD levels during our measurement protocol were similar to those commonly experienced in the open environments occupied by some of our study species and to those less commonly experienced during sunflecks in closed canopy sites.

Gas exchange

To examine the use of light energy downstream of the photosystems, we measured maximum photosynthetic rates (A_{max}) in all our species in both the field and the greenhouse using a portable gas exchange system (LI-6400; Li-Cor, Lincoln, NE, USA). A_{max} was sampled on the newest fully expanded leaf of five individuals of each species. For detailed methods on gas exchange see Givnish *et al*. (2004).

In vivo *estimates of xanthophyll-cycle pigments*

We used a spectral reflectance method developed by Gamon *et al*. (1997) and Gamon and Surfus (1999) to provide a general estimate of the pool size of xanthophyll-cycle pigments *in situ*. This method measures the ratio of reflectance at 531 nm to that at 570 nm, also known as the photochemical reflectance index (PRI), under dark and light conditions. The PRI has been widely used in remote sensing as a proxy of radiation use efficiency, a critical parameter for remote sensing of photosynthesis (Grace *et al*. 2007).

We chose the newest fully expanded leaf on five individuals of each species. Leaves were acclimated in the dark for at least 1 h before measurement. After dark acclimation, the leaves were illuminated and within 1 s of illumination a spectral scan was made at $t = 0$. Subsequent scans were made at $t = 5$ min and $t = 10$ min, after which time the spectral signature did not exhibit change at the focal wavelengths. We calculated the change in the photochemical reflectance index (Δ PRI) following Gamon and Surfus (1999) as PRI ($t = 0$) − PRI ($t = 10$), where

$$PRI = (R_{531} - R_{570}) : (R_{531} + R_{570}) \quad (1)$$

In this equation, R is reflectance and the subscript numbers indicate the spectral band. The change in spectral signal is related to the conversion of violaxanthin to zeaxanthin. Δ PRI represents an estimate of the total zeaxanthin formed during the dark to light transition. Although we did not calibrate our Δ PRI to the actual levels of xanthophyll-cycle pigments, previous studies suggest that they represent a good relative index of xanthophyll-cycle pigments (Gamon *et al*. 1997; Gamon and Surfus 1999) and, thus,

an indirect way to look at the mechanisms underlying species differences in response to light stress.

Statistical analysis

We used a one-way ANOVA (species) for the field data and a two-way ANOVA (species × light treatment) for the greenhouse data. The greenhouse ANOVA examined species × environment interactions. We only had data from two individuals of *Cyanea leptostegia* A.Gray for Δ PRI, so this species was excluded from the analyses that involved that variable. We also examined correlations among mean functional and fluorescence trait values and light for field populations. Mean functional trait values (e.g. leaf lifespan, respiration rates, SLA and N) were drawn from previously published work (Givnish *et al*. 2004).

Results

Habitat sunfleck activity

Sunfleck length and daily minutes were significantly related to estimates of integrated light availability at our sites (Table 1) (Givnish *et al*. 2004; Montgomery and Givnish 2008). Habitats with low daily PFD totals had short sunflecks compared with sunnier habitats. The average sunfleck length varied from 1.4 ± 1.7 min for *Cyanea floribunda* E.Wimm. growing in shaded forest understories to 31.2 ± 2.1 min for *Trematolobelia kauaiensis* (Rock) Skottsb. growing on open ridges (Table 1). The average number of minutes of sunflecks per day varied from 31 ± 21 min day^{-1} for *C. floribunda* to 533 ± 59 min day^{-1} in *Lobelia villosa* (Rock) St. John & Hosaka, a species that grows in open alpine bog sites (Table 1). Despite strong differences in the average length and total minutes of sunflecks, it appears that all species can experience quite long sunflecks (Table 1). Even the most shaded species, *C. floribunda*, occasionally experienced sunflecks as long as 21 min.

F_v/F_m, *NPQ*, Δ *PRI and recovery in the field*

We found no significant differences among species in dark-acclimated F_v/F_m under field conditions (Table 2). The average F_v/F_m across the 11 species was 0.81 ± 0.005

Table 2.   *In situ* chlorophyll fluorescence and leaf spectral characteristics of 11 species of Hawaiian lobeliads
Superscript letters indicate means that differ significantly from each other ($P < 0.05$). Species are ordered from lowest to highest daily photon flux density (PFD).
F_v/F_m, maximum efficiency of PSII; NPQ, non-photochemical quenching; Δ PRI, change in the photochemical reflectance index

Species	F_v/F_m		Percentage recovery of F_v/F_m		NPQ of F_m'		Δ PRI	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Cyanea floribunda	–	–	–	–	–	–	0.010[a]	0.0020
Cyanea pilosa	0.816[a]	0.012	75[a]	6.7	3.2[a]	0.6	0.018[a]	0.0045
Cyanea hirtella	0.850[a]	0.012	77[a]	6.7	5.8[b]	0.2	0.045[bcd]	0.0048
Cyanea leptostegia	0.813[a]	0.012	84[a]	6.7	3.8[a]	0.8	–	–
Clermontia parviflora	0.800[a]	0.010	82[a]	5.3	4.5[ab]	0.2	0.030[ab]	0.0038
Lobelia yuccoides	0.808[a]	0.012	71[a]	6.7	4.0[a]	0.3	0.026[ab]	0.0007
Delissea rhytidosperma	0.796[a]	0.012	71[a]	6.7	4.0[ab]	0.1	0.046[bcd]	0.0041
Clermontia fauriei	0.819[a]	0.012	71[a]	6.7	5.0[ab]	0.4	0.063[d]	0.0054
Trematolobelia kauaiensis	0.812[a]	0.014	80[a]	7.5	5.0[ab]	0.1	0.055[cd]	0.0049
Lobelia villosa	0.794[a]	0.010	70[a]	6.2	4.3[ab]	0.1	0.047[bcd]	0.0065
Brighamia insignis	0.798[a]	0.014	70[a]	7.5	4.3[ab]	0.7	0.040[bc]	0.0020

(mean ± s.e.). This number is well within the narrow range of values expected for unstressed plants (Björkman and Demmig 1987). In addition, we did not find any significant differences among species in the trajectory of recovery from a 20-min high light stress (PFD = 2500 µmol m^{-2} s^{-1}; Fig. 1). All species recovered an average of 74 ± 1.4% (mean ± s.e.) of maximal F_v/F_m after 40 min in dark conditions (Table 2). Species did differ significantly in the NPQ of F_m' during the high light stress ($F_{9,29}$ = 2.44, P < 0.01; Table 2), but the values were all relatively high, ranging from 3.2 (± 0.6) to 5.8 (± 0.2). Species also differed significantly in Δ PRI (Table 2; $F_{9,34}$ = 17.13, P < 0.0001). The lowest Δ PRI values occurred in *C. floribunda* (0.010 ± 0.002) and *Cyanea pilosa* A.Gray subsp. *longipedunculata* (Rock) Lammers (0.018 ± 0.0045), species from the shadiest habitats. We found the highest Δ PRI levels in *Clermontia fauriei* H.Lév. (0.063 ± 0.0054), a species from high light habitats (Table 2).

Trait correlations and relationship to the light environment

Photosynthetic capacity (A$_{max}$) was not related to any of the fluorescence traits or to Δ PRI. We did find a positive correlation between NPQ and area-based respiration rates (r = 0.75, P = 0.03) and between F_v/F_m and leaf lifespan (r = 0.61, P = 0.06). Neither F_v/F_m nor leaf lifespan were related to the light environment. Δ PRI, an estimate of zeathathin conversion in dark to light transitions, and NPQ, an estimate of non-photochemical energy dissipation, were strongly positively correlated (Fig. 2; r = 0.82, P = 0.01) and both increased with the natural log of average sunfleck length in a species habitat (Fig. 3). Δ PRI and NPQ were also negatively correlated with SLA (r = −0.76,

P = 0.03 and r = −0.78, P = 0.02, respectively). Our prior work has shown that A$_{max}$ increases and SLA decreases with increasing light availability (cf. fig. 3 and table 4 in Givnish *et al.* (2004)).

F_v/F_m in the greenhouse

Although the field populations showed no significant differences in dark-acclimated F_v/F_m or recovery, we did find significant differences among the species in both traits across the four greenhouse light treatments (Fig. 4; Table 3). *Clermontia parviflora* Gaudich. Ex A.Gray, from wet forest gaps and edges, had the lowest average F_v/F_m, whereas *Brighamia insignis* A.Gray, a species from open cliff habitats showed the highest average F_v/F_m (Fig. 5A). All species showed significant decreases in F_v/F_m as the light environment increased (Table 3; Fig. 5B). There was no significant interaction between species and light treatment, suggesting that species differences result from genetic divergence among the taxa studied. In general, the patterns of recovery after light stress mirrored the trends in dark-acclimated F_v/F_m (Fig. 5C, D; Table 3). Species with high dark-acclimated F_v/F_m recovered more rapidly after high light stress. Recovery ranged from 60% of pre-stress levels in *C. parviflora* in the 80% light treatment to 94% of pre-stress levels in *B. insignis* growing in the 35% light treatment (Fig. 5C). Across species, plants in the open light treatment had significantly lower rates of recovery (Fig. 5D; Table 3).

Discussion

Adaptive radiation – the rise of a diversity of ecological roles and attendant adaptations – is a fundamental process linking ecology
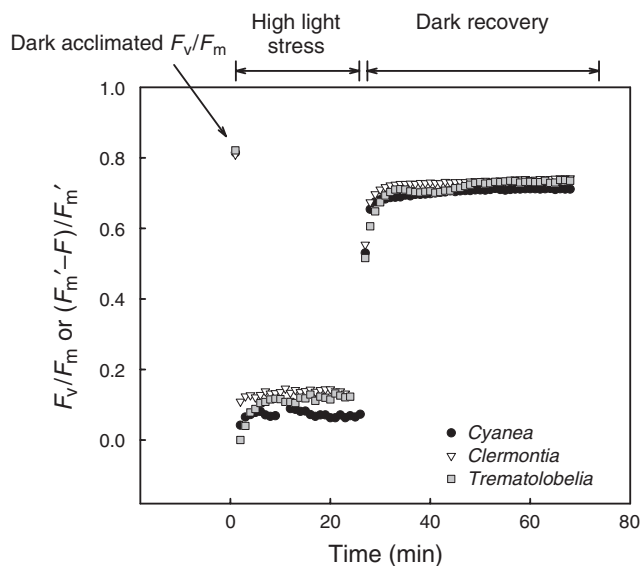

Fig. 1. Maximum efficiency of PSII (dark-acclimated F_v/F_m), $(F_m'-F)/F_m'$ during a 20-min high light stress (2500 umol m^{-2} s^{-1}) and recovery of F_v/F_m during a subsequent 40-min dark period. Values are shown for individuals of three species representative of low, medium and high light environments: *Cyanea pilosa* var. *longipedunculata* (PFD = 3.2 mol m^{-2} day^{-1}), *Clermontia parviflora* (PFD = 8.3 mol m^{-2} day^{-1}) and *Trematolobelia kauaiensis* (PFD = 21.1 mol m^{-2} day^{-1}).
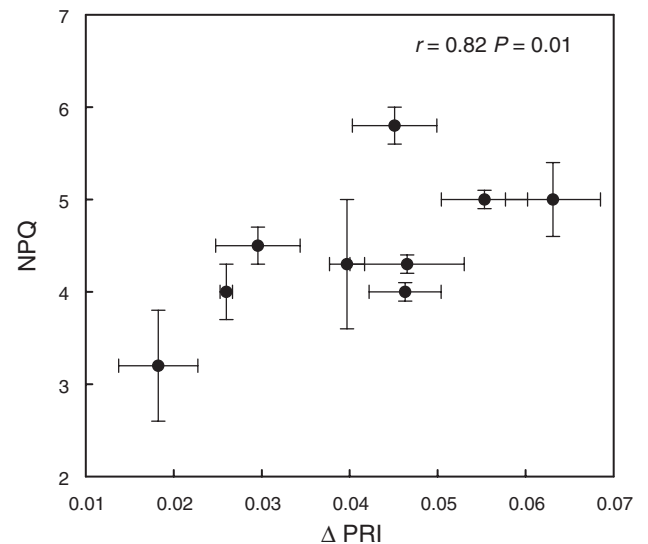

Fig. 2. Relationship between non-photochemical quenching (NPQ) and a change in the photochemical reflectance index (Δ PRI) for nine respresentative species of Hawaiian lobeliads. Δ PRI was used as a proxy for the levels of de-epoxidised xanthophyll-cycle components formed during dark to light transitions. Dots and errors bars represent the mean ± s.e. of fluorescence data for 4–8 individuals of each species. *Cyanea leptostegia* and *Cyanea floribunda* had missing data and were excluded. From left to right the species are *Cyanea pilosa* var. *longipedunculata*, *Lobelia yuccoides*, *Clermontia parviflora*, *Brighamia insignis*, *Cyanea hirtella*, *Delissea rhytidosperma*, *Lobelia villosa*, *Trematolobelia kauaiensis* and *Clermontia fauriei*.
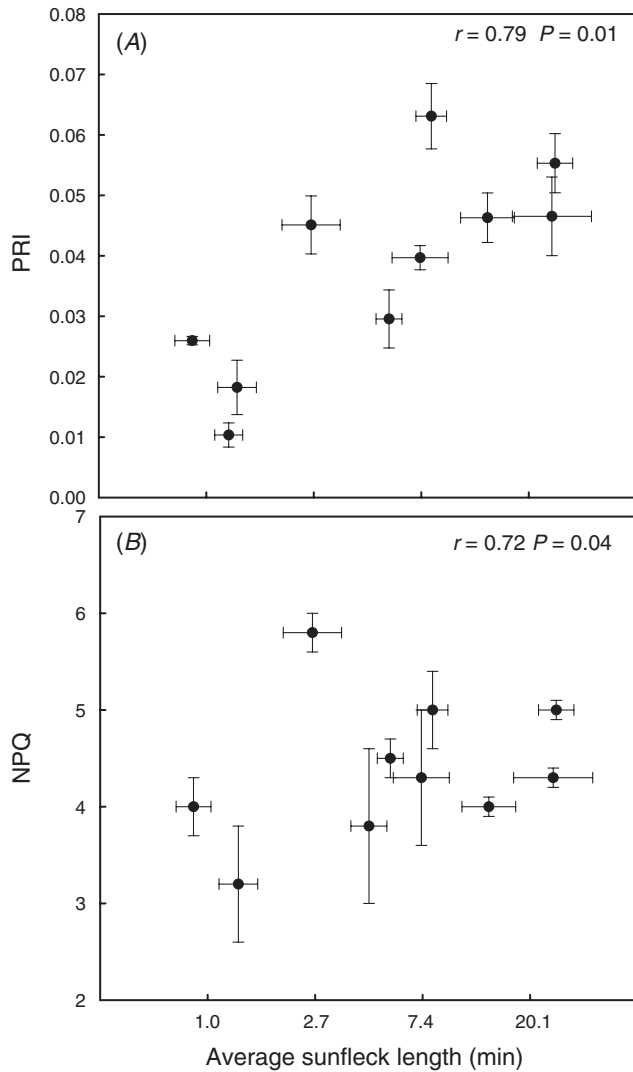
Fig. 3. (*A*) Change in the photochemical reflectance index (Δ PRI) and (*B*) non-photochemical quenching (NPQ) of F_m' as a function of the average length of sunflecks experienced by 10 representative species of Hawaiian lobeliads. Δ PRI was used as a proxy for the levels of de-epoxidised xanthophyll-cycle components formed during dark to light transitions. Dots and errors bars represent the mean ± s.e. of spectral or fluorescence data for 4–8 individuals of each species and the mean ± s.e. of photon flux density (PFD) measurements for all datalogged individuals of each species. *Cyanea leptostegia* is missing from (*A*) and *Cyanea floribunda* is missing from (*B*). From left to right the species are *Lobelia yuccoides*, *Cyanea floribunda*, *Cyanea pilosa* var. *longipedunculata*, *Cyanea hirtella*, *Cyanea leptostegia*, *Clermontia parviflora*, *Brighamia insignis*, *Clermontia fauriei*, *Delissea rhytidosperma*, *Lobelia villosa* and *Trematolobelia kauaiensis*.

Fig. 4. Maximum efficiency of PSII (dark-acclimated F_v/F_m), $(F_m'-F)/F_m'$ during a 20-min high light stress (2500 umol m^{-2} s^{-1}) and recovery of F_v/F_m during a subsequent 40-min dark period. Values are shown for individuals of *Clermontia parviflora* grown in four common-garden greenhouse light treatments. Low, 6% full sun and 0.53 red : far red (R : FR); medium, 14% full sun and 0.62 R : FR; high, 32% full sun and 0.72 R : FR; open, 80% full sun and 0.83 R : FR.

and evolution (Givnish 1986). Adaptive radiations are characterised by rapid diversification in species that share a common ancestry, correlation of environmental variation with phenotypic divergence and the rise of traits that are functionally significant to fitness in the new environments (Schluter 2000). Although there have been tremendous advances in our knowledge
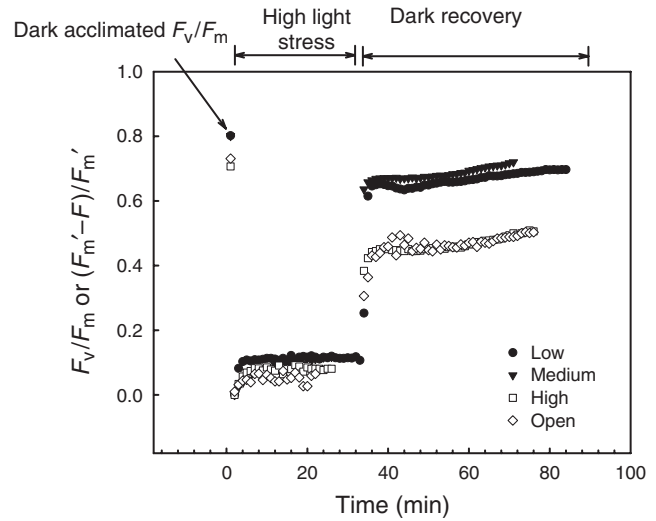
of the origin and phylogeny of Hawaiian plant radiations (Gillespie *et al*. 1994; Givnish *et al*. 1995; Shaw 1996; Piano *et al*. 1997; Ganders *et al*. 2000; Lindqvist *et al*. 2003; Nepokroeff *et al*. 2003; Rundell *et al*. 2004; Schneider *et al*. 2005), for almost all of these radiations we lack the functional data necessary to understand the ecological significance of the phenotypic divergences observed. This information is fundamental to determining whether a radiation is indeed adaptive. A body of work has developed on functional trait variation in Hawaiian plant radiations, most notably on *Euphorbia* and *Scaevola* (Robichaux and Pearcy 1980; Pearcy *et al*. 1982; Robichaux and Pearcy 1984), the silversword alliance (Robichaux 1984; Robichaux and Canfield 1985; Robichaux *et al*. 1990), and *Schiedea* (Sakai *et al*. 1997; Weller *et al*. 1998). Our work on the Hawaiian lobeliads expands the scope of these prior studies by examining habitat divergence and accompanying functional diversification in field populations and under multiple common-garden greenhouse light treatments. We have shown that the lobeliads have undergone an adaptive radiation in photosynthetic physiology that is strongly related to the light regime (Givnish *et al*. 2004; Montgomery and Givnish 2008). In the present study, we have shown that, despite invasion into progressively more shady habitats, the Hawaiian lobeliads retain the ability to recover from high light stress at the magnitude of long sunflecks (Table 2). High recovery rates might be related to increasing levels of xanthophyll-cycle pigments with light availability and high levels of NPQ during exposure to high light in all species. However, species differ in their physiological resilience to long-term light stress (e.g. those encountered in our common-garden treatments). Species native to high light habitats were more resistant to light stress than those from low light habitats (Table 3; Figs 4 and 5).
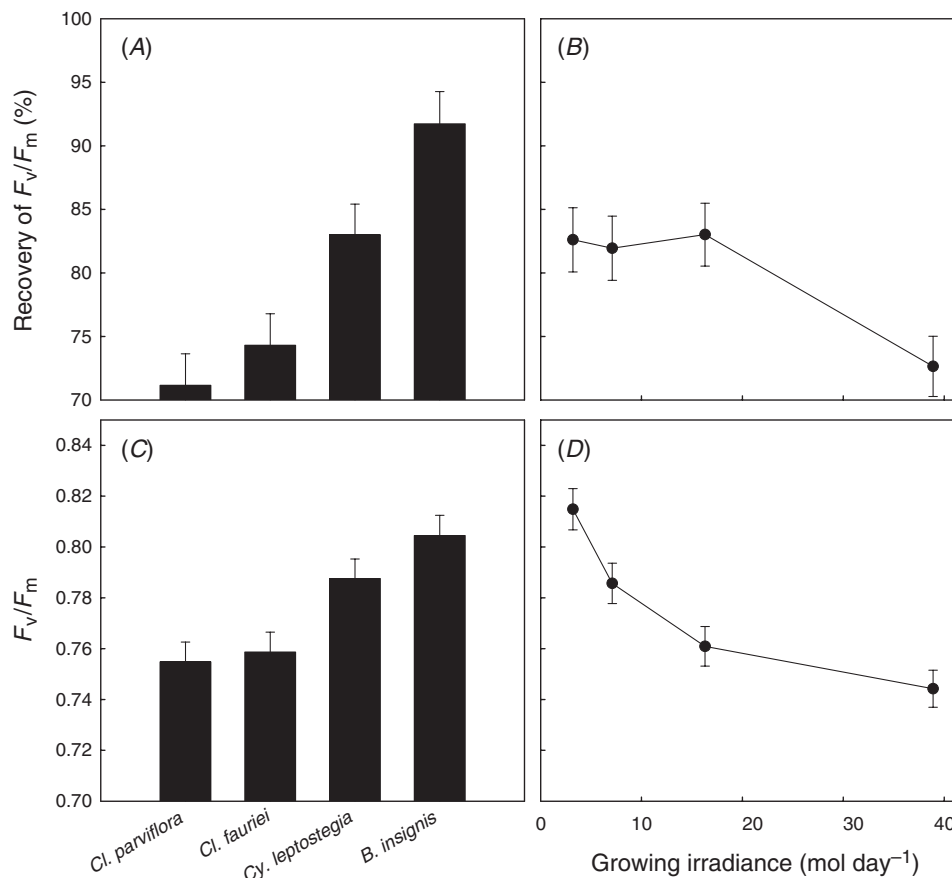
Fig. 5. Maximum efficiency of PSII (dark-acclimated F_v/F_m) and the percentage recovery of F_v/F_m in *Cyanea leptostegia*, *Clermontia parviflora*, *Clermontia fauriei* and *Brighamia insignis* grown in four common-garden greenhouse light treatments (Low, 6% full sun and 0.53 red : far red (R : FR); medium, 14% full sun and 0.62 R : FR; high, 32% full sun and 0.72 R : FR; open, 80% full sun and 0.83 R : FR). (*A*) Species means for $F_v/F_m \pm$ s.e., (*B*) treatment means $\pm$ s.e. for F_v/F_m, (*C*) species means $\pm$ s.e. for percentage recovery and (*D*) treatments means $\pm$ s.e. for percentage recovery. Species in (*A*) and (*B*) are ordered by native habitat photon flux density (PFD) from lowest to highest (left to right), except for *Cyanea leptostegia*, which as an adult occurs in much brighter microsites than those measured for its saplings.

Table 3. ANOVA results for chlorophyll fluorescence of four lobeliads species (*Cyanea leptostegia, Clermontia parviflora, Clermontia fauriei* and *Brighamia insignis*) growing in four common-garden greenhouse light treatments (Low, 6% full sun and 0.53 red : far red (R : FR); medium, 14% full sun and 0.62 R : FR; high, 32% full sun and 0.72 R : FR; open, 80% full sun and 0.83 R : FR) in a shadehouse at the Volcano Agricultural Experiment Station, Volcano, Hawaii
F_v/F_m, maximum efficiency of PSII

Source		F_v/F_m				Percentage recovery of F_v/F_m		
	d.f.	MS	F	P	d.f.	MS	F	P
Species	3	0.01210	9.17	<0.0001	3	1755.0	13.697	<0.0001
Treatment	3	0.02066	15.66	<0.0001	3	545.0	4.254	0.0082
Species × treatment	9	0.00117	0.89	0.5388	9	164.0	1.280	0.2638
Error	72	0.00132			68	128.1		

Photoprotection in the field

As expected, our data corroborate earlier findings that most field-grown plants show similar dark-acclimated F_v/F_m unless severely stressed (Björkman and Demmig 1987). However, our hypothesis

that species from high light habitats would recover more rapidly from high light stress was not supported. The short, high light stress applied *in situ* did not lead to significant differences among species in recovery of F_v/F_m after stress (Fig. 1; Table 2). This

result contrasts with previous work on a native Hawaiian fern that showed reduced short-term recovery of F_v/F_m when compared with an invasive fern (Durand and Goldstein 2001). It also does not support the idea that sunflecks can lead to significant declines in PSII function in shade-adapted species, but instead suggests rapidly reversible photoprotection under dynamic sunfleck regimes (Logan *et al.* 1997; Adams *et al.* 1999).

The uniform ability to recover from a short, high light stress across species that differ in their light environment might be related to the nature of the sunfleck regimes found in Hawaiian forests. Hawaiian forests appear to be brighter than other tropical forests (Givnish *et al.* 2004; Montgomery and Givnish 2008) and have correspondingly longer sunflecks (see Chazdon 1988; Lee 1989; Valladares *et al.* 1997). Indeed, species from the lowest PFD regimes in the present study can experience flecks as long as 20 min, the length of the experimental 'stress' we applied.

The high levels of post-stress recovery might also be related to traits associated with how species use incoming light. As habitat light availability increased, we found evidence that species had increased levels of both photochemical and non-photochemical energy dissipation (Table 2; Fig. 3; Givnish *et al.* 2004). Photosynthetic capacity and NPQ increased significantly with light availability. Moreover, the levels of NPQ found across all our species were quite high, ranging from 3.8 to 5.8. These levels are higher and from a narrower range than those found in a previous study of sun-exposed leaves of 24 species (~1.5–5) from life-forms as different as trees and eggplants (Demmig-Adams and Adams 1996*b*). The levels are similar to the levels found in open-grown *Opuntia macrorhiza* Engelm., a cactus from Colorado, USA (Barker and Adams 1997).

In situ *estimates of xanthophyll-cycle pigments track habitat variation*

The carotenoids of the xanthophyll cycle have been shown to play an important role in non-photochemical dissipation of excess energy (Demmig-Adams *et al.* 1995; Demmig-Adams and Adams 1996*a*, 1996*b*; Logan *et al.* 1998*b*). High levels of de-epoxidised xanthphyll-cycle components correlate strongly with NPQ within and among species (Demmig-Adams and Adams 1996*b*). In the present study, the strong relationship between Δ PRI and NPQ (Fig. 3) provides indirect support for the role of xanthophyll-cycle pigments in the photoprotective response to light in our study species.

Traditionally, xanthophyll levels are assessed via the destructive analysis of leaf tissue using HPLC. However, there is growing interest in remotely sensing leaf carotenoids and other leaf pigments for use in regional and global models of photosynthesis. The photochemical reflectance index has emerged as a potential tool to remotely quantify leaf carotenoids, particularly those related to the xanthophyll cycle. Moreover, in endangered species research there is strong pressure to identify non-destructive approaches for measuring plant stress and resistance to stress. Our use of Δ PRI moves beyond characterising pigments to assessing function. The relationship we found between Δ PRI and NPQ suggests that levels of non-photochemical energy dissipation, important for reducing light stress, can be detected non-destructively in these species using leaf spectral reflectance.

We found a strong positive relationship between Δ PRI and habitat light availability, suggesting a diversification within the lobeliad lineage in the levels of photoprotective leaf carotenoids, similar to those seen in field studies of single species or phylogenetically disparate species (Bilger and Björkman 1990; Brugnoli *et al.* 1994; Logan *et al.* 1996; Logan *et al.* 1998*b*; Niinemets *et al.* 1998). Moreover, the functional link to habitat light availability is supported by the correlations between Δ PRI, NPQ and leaf traits characteristic of high light environments (e.g. high SLA and high respiration rates). The diversity of field responses might result from both acclimation and adaptation of photoprotective processes in the lobeliad lineage.

Common-garden support for diversification in the stress response

Although field studies provide key insights into the operating values of traits, they cannot distinguish between environmental and genetic sources of trait variation. Nor can our field studies tell us much about trait plasticity, given that any single species of Hawaiian lobeliad occurs across a narrow range of light conditions. A common-garden approach is needed to gain insight into the interaction of genetic and environmental controls on trait variation. Our common-garden greenhouse study provided key information on trait variation among species and light environments. We found three major trends (Fig. 5): (1) a reduction in F_v/F_m and to a lesser extent percentage recovery as treatment PFD increased, (2) species differences in F_v/F_m and percentage recovery that parallel differences in native habitat PFD, and (3) no evidence of an interaction between species and treatment PFD.

Lower values of F_v/F_m across all species as growth PFD increases might be related to downregulation of PSII or photoinactivation of PSII, both of which can serve a photoprotective function (Öquist *et al.* 1992; Lee *et al.* 2001). Despite the potential negative effects of excess light, considerable inactivation of PSII can occur without a significant effect on carbon assimilation under high light conditions (Lee *et al.* 1999; Kornyeyev *et al.* 2006). A reduction in F_v/F_m (Fig. 5B) does not appear to reduce the short-term recovery of PSII function, except in the highest light treatment (Fig. 5D). The reduction in percentage recovery in the highest light treatment might result from a combination of high light and temperature in this treatment. There is evidence that simultaneous high PFD and high temperatures result in lower F_v/F_m and slower recovery (Gamon and Pearcy 1990; Mulkey and Pearcy 1992; Valladares and Pearcy 1997). For example, *Vitis californica* [Parry] can partially or completely recover overnight from high light or high temperature stress. However, the combined stress leads to sustained declines in F_v/F_m. Similarly, recovery of F_v/F_m after light stress in the understory herb *Alocasia macrorhiza* Schott is inhibited at high temperatures (Mulkey and Pearcy 1992).

The second trend, suggesting that native habitat is related to level of PSII function, supports our hypothesis that species from high light habitats will show higher levels of PSII function in the face of sustained high light levels and show more rapid recovery after stress. However, the small sample size ($n = 4$ species) does limit the generality of this result. *B. insignis* occurs on open, relatively dry cliff faces that receive an average of

30 mol m^{-2} day^{-1} of PFD. *Brighamia*'s dark-acclimated F_v/F_m averaged 0.80 ± 0.01 across all light treatments and recovered >90% of PSII function within 40 min in the dark. In contrast, *C. parviflora*, a species found in wet forest edges and gaps (with an average daily PFD of 8.3), had significantly lower dark-acclimated F_v/F_m and recovered only ~70% of PSII function within 40 min. *C. leptostegia*, from the lowest light environment, appears somewhat anomalous. However, unlike all other species studied, *C. leptostegia* occupies a radically different light environment as an adult, when it occurs in the canopy or subcanopy of *Acacia koa* A.Gray forests, than as a juvenile, when it occupies shaded understories whose PFD we characterised. Therefore, it is found in 'full sun' conditions as an adult and our field measurements of PFD – constrained to shorter subdominant and sapling individuals by the difficulty of access to dominant crowns – almost surely underestimate the native PFD for *C. leptostegia* over its life cycle.

Finally, across the PFD treatments in our common-garden study, species maintain their rank order of response in both F_v/F_m and percentage recovery (Fig. 5*A*, *C*). Thus, although species show considerable plasticity in photoprotection across the common-garden light conditions, the lack of a species × environment interaction suggests that much of the observed variation among species is independent of the growth environment. This suggests the possibility of a diversification in photoprotective traits and provides further evidence for functional diversification in the Hawaiian lobeliads.

Our analysis extends prior research by demonstrating a strong quantitative relationship between photoprotective energy dissipation via the xanthophyll cycle and growth light environments across a large group of closely related species (Figs 2 and 3). Furthermore, the observed shifts among species in photochemistry, NPQ and xanthophyll-cycle pigments tend to maintain long-term PSII function across a broad range of field environments through increasing levels of photoprotection in species that typically occupy the sunniest environments. These results parallel our findings for steady-state and dynamic photosynthetic light responses (Givnish *et al.* 2004) and provide additional evidence for the potentially adaptive diversification of photosynthetic physiology in the Hawaiian lobeliads.

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