

1 **Photonic multilayer structure of *Begonia* chloroplasts enhances**
2 **photosynthetic efficiency**
3

4 Matthew Jacobs^{1#}, Martin Lopez-Garcia^{2#}, O-Phart Phrathep¹, Tracy Lawson³,
5 Ruth Oulton², Heather M. Whitney^{1*}

6 ¹School of Biological Sciences, University of Bristol, Life Sciences Building, Tyndall
7 Avenue, Bristol BS8 1TQ, UK.

8 ²Department of Electrical and Electronic Engineering, University of Bristol, Bristol BS8
9 1TH, UK.

10 ³School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4
11 3SQ, UK

12 # Joint first author

13 *Correspondence to: heather.whitney@bristol.ac.uk
14

15 Enhanced light-harvesting is an area of interest for optimising both natural photosynthesis and
16 artificial solar energy capture^{1,2}. While iridescence has been shown to exist widely and in diverse
17 forms in plants and other photosynthetic organisms and symbioses^{3,4}, there has yet to be any
18 direct link demonstrated between iridescence and photosynthesis. Here we show that epidermal
19 chloroplasts, also known as iridoplasts, in shade-dwelling species of *Begonia*⁵, notable for their
20 brilliant blue iridescence, have a photonic crystal structure formed from a periodic arrangement
21 of the light-absorbing thylakoid tissue itself. This structure enhances photosynthesis in two ways:
22 by increasing light capture at the predominantly green wavelengths available in shade conditions,
23 and by directly enhancing quantum yield by 10-15% under low light conditions. These findings
24 together imply that the iridoplast is a highly modified chloroplast structure adapted to make best
25 use of the extremely low light conditions in the tropical forest understory in which it is found^{5,6}.
26 A phylogenetically diverse range of shade-dwelling plant species have been found to produce
27 similarly structured chloroplasts⁷⁻⁹, suggesting that the ability to produce chloroplasts whose

28 membranes are organized as a multilayer with photonic properties may be widespread. In fact,
29 given the well-established diversity and plasticity of chloroplasts^{10,11}, our results imply that
30 photonic effects may be important even in plants that do not show any obvious signs of
31 iridescence to the naked eye but where a highly ordered chloroplast structure may present a clear
32 blue reflectance at the microscale. Chloroplasts are generally thought of as purely
33 photochemical; we suggest that one should also think of them as a photonic structure with a
34 complex interplay between control of light propagation, light capture, and photochemistry.

35

36 Photonic crystals are periodic nanoscale structures which interact with light, resulting in a
37 number of optical phenomena. In artificial systems, these photonic properties have been
38 investigated for their light harvesting properties as they can strongly enhance the performance of
39 devices for solar energy production, through either light-trapping¹ or slow light mechanisms².
40 However, one group of photonic crystals that have repeatedly been associated with light
41 harvesting roles including optimised light distribution within photosynthetic tissues³, but which
42 have yet to be directly linked to enhanced light harvesting, are natural biological photonic
43 crystals. Photonic structures are widespread in nature, where they are typically associated with
44 structural colour^{4,12,13}. Although more extensively researched in animals, photonic effects have
45 further implications in plants given the importance of light manipulation for photosynthesis⁴. A
46 striking example of structural colour in plants is presented by the iridescent blue leaves observed
47 in a diverse range of tropical plant species adapted to deep forest shade conditions, however little
48 direct evidence of the function of this structural colour has been presented^{14,15}.

49

50 Blue iridescent leaf colouration has been reported in the genus *Begonia* and explored in a single
51 species, *B.pavonina*¹⁶. Unusual plastids containing highly ordered internal structures termed
52 iridoplasts were observed in the adaxial epidermis and proposed as the source of the blue
53 colouration⁵. However, it has never been demonstrated whether iridoplasts are indeed
54 responsible for this blue structural colouration, or what biological function it signifies.

55

56 To investigate *Begonia* leaf iridescence directly, we imaged leaves of *B.grandis* × *pavonina*. This
57 hybrid was generated and used for the majority of work as it displays the intense iridescence
58 typical of *B.pavonina* whilst maintaining the more vigorous growth habit typical of *B.grandis*.
59 Figure 1a shows the vivid iridescence observed in the mature leaves of this hybrid. Using
60 reflected light microscopy, we imaged (Fig. 1b) and measured spectra (Fig. 1d) from single
61 iridoplasts *in vivo*. Both confirm that iridoplasts show a strong blue peak in reflectance observed
62 at central wavelength $\lambda_c \approx 470$ nm with a spectral width of ~ 60 nm. Both TEM (Supplementary
63 Fig. S1) and freeze-fracture cryo-SEM (Fig. 1c) clearly show regularly spaced grana. Iridoplasts
64 were observed in all adaxial epidermal cells of a phylogenetically diverse selection of *Begonia*
65 species, including in leaves where iridescence was not visible to the naked eye. TEM further
66 confirmed the presence of the highly ordered iridoplasts in these species (Supplementary
67 Fig. S2). Despite some variation in spectral width, both the observed colour and corresponding λ_c
68 is similar for all iridoplasts in all species measured to date, with no observed reflectance out of
69 the blue-turquoise region ($450 < \lambda_c < 500$ nm). We also confirmed that both the iridoplasts and
70 archetypal mesophyll chloroplasts ('chloroplast' from herein) show similar variation in
71 autofluorescence (Supplementary Fig. S3) indicating that iridoplasts may show a photosynthetic
72 function.

73

74 To explore the possible biological function, we examined the micro and nanoscopic morphology
75 of the iridoplast in quantitative detail to obtain input data for an optical model of the absorption
76 and reflectance of the structure. Chloroplast structure is well established (Supplementary
77 Fig. S7,^{10,17}) however in iridoplasts only a few thylakoids (three in Fig.2a) are stacked into grana
78 of ~ 40 nm thickness, that are themselves regularly spaced by d_s ~100 nm. Ultrastructural
79 analysis revealed that each granum is formed from the same number of thylakoids (N_m)
80 throughout the iridoplast (Supplementary Fig.4). The average number of thylakoids per granum
81 can vary with $N_m = 3.3 \pm 0.8$ between iridoplasts. Fast Fourier Transform (FFT) imaging
82 (Supplementary Fig. S5) allows an accurate estimate of the total period between grana to be $\Lambda =$
83 170 ± 20 nm. The thickness of the thylakoid membrane ($M/2$) and the lumen (L) were estimated
84 from the TEM to be $M = 6.68 \pm 0.76$ nm and $L = 7.52 \pm 0.82$ nm respectively.

85

86 These data were used as input into an optical transfer matrix method (TMM) model (see
87 Methods) that calculates electric field intensity and reflectance for multiple periodic layers of
88 varying refractive index (Fig. 2a). The reflectance spectra of single iridoplasts were analysed
89 and compared with that predicted by our model (Fig. 2b). A high level of replication for the
90 spectral shape of the reflectance is obtained with values of $d_s = 125, 115$ nm. To establish if this
91 model predicts the formation of a photonic stopband within the observed thickness parameters
92 we modelled a variation of d_s over a wide range of values from $d_s = 0$ to 250 nm for a constant
93 number of grana ($N = 8$). As shown in Fig. 2c, no strong reflectance at any wavelength is
94 observed when $d_s = 0$, mimicking chloroplasts with partition gaps between grana of a few
95 nanometers¹⁸. However in the range $100 < d_s < 160$ nm a strong reflectance peak appears at short

96 wavelength for small d_s ($\lambda \approx 430$ nm) and at long wavelengths for long d_s ($\lambda \approx 560$ nm). This is a
97 clear indication that the chlorophyll pigments in the thylakoid membranes are capable not only of
98 absorption but also sufficient modification of the real part of the refractive index to produce a
99 photonic stop-band. The data also indicate the approximate range of wavelengths over which one
100 might see peak reflectance (430-560 nm). Measurement of 20 iridoplasts (Supplementary Fig.
101 S6) confirms a range of peak reflectance from ~ 435 -500 nm, towards the blue end of the
102 calculated range. Finally, we also performed angular reflectance measurements to evaluate the
103 angular dependence of the iridoplast optical properties. As expected from a multilayer structure
104 we obtained a blueshift of the reflectance peak as the angle of incidence is increased
105 (Supplementary Fig. S6). Comparison with calculations using the optical model described before
106 also shows a good agreement.

107 This model was used to evaluate the effect of iridoplast photonic structure on absorption at the
108 thylakoid membranes. Photonic crystals can strongly enhance or reduce absorption depending
109 on absorber spatial position and spectral response. The mechanism by which light-matter
110 interaction leads to enhanced absorptance when the spacing period is close to the wavelength of
111 light in the structure is known as “slow light”. This phenomenon arises from the reduction of the
112 group velocity of the light propagating through the photonic crystal for those wavelengths within
113 the photonic band edge¹⁹, in our case $\lambda \approx 440$ nm and $\lambda \approx 520$ nm (Fig. 2). When light propagates
114 through a photonic crystal at, or close to the stop-band, interference of forward and backward
115 propagating light leads to the formation of a standing wave with nodes (low $|E|^2$) and antinodes
116 (high $|E|^2$) at specific locations. Interestingly it is for the longer wavelength edge of the photonic
117 band where the electric field is concentrated in the high refractive index layers, in our case, the
118 thylakoid membranes. The absorptance will then be enhanced for those wavelengths for which

119 the slow light phenomena occurs simultaneously with the appropriate positioning of the
120 photosynthetic membranes within the iridoplast. In general terms it is established that while
121 absorption is reduced for those wavelengths within the photonic band gap (usually shown as
122 strong reflectance in natural photonics) it can be strongly enhanced at the band gap edges¹⁹. We
123 calculated the absorptance of the organelles as $A^{Ir}(\lambda) = I - R(\lambda) - T(\lambda)$ ², where R and T are the
124 reflectance and transmittance of the multilayer at wavelength λ respectively. Using the previous
125 structural parameters we compared A^{Ir} with the absorptance of the same structure (A^{Chlo}) without
126 partition gap between grana ($d_s = 0$, *i.e.* similar to an unmodified chloroplast²⁰), defining the
127 absorptance enhancement factor as the ratio $\gamma(\lambda) = A^{Ir}(\lambda)/A^{Chlo}(\lambda)$. One obtains an absorptance
128 enhancement ($\gamma > 1$) for all wavelengths in the green-red spectral range ($500 < \lambda < 700\text{nm}$) and a
129 reduction ($\gamma < 1$) in the blue ($\lambda < 500\text{ nm}$) as seen in Fig. 3a. The cut-off wavelength for $\gamma > 1$ will
130 depend on d_s , with two values for d_s plotted (125nm, 115nm). In fact, single wavelength analysis
131 unveils maximum enhancements (reductions) of $\gamma = 1.27(0.73)$ for $\lambda = 523$ (466) nm and
132 $d_s = 125\text{ nm}$. Fig. 3b shows a calculation of the spatial distribution of the light intensity $|E|^2$
133 across the grana and stroma. Fig. 3b also shows the position of the grana with respect to the
134 antinodes for different wavelengths. For blue light (460 nm), the grana are located at the nodes,
135 while for 530 nm (green) the grana are located at antinodes. This explains intuitively why the
136 calculations in Fig. 3a show relatively more absorptance in the green and less in the blue spectral
137 regions. The angular dependency of the iridoplast optical properties could also influence the
138 absorptance. Therefore, we studied the angular dependence of γ (Fig. 3c). As can be observed,
139 the region where $\gamma > 1$ (absorptance enhancement) extends to incident angles $\theta < 30^\circ$.
140 Interestingly the angular dependence for γ is not very strong showing an almost constant
141 maximum value $\gamma \approx 1.2$ for $500 < \lambda < 550\text{ nm}$. This would support our hypothesis of the blue

142 coloration being a by-product of the light harvesting functionality of the iridoplast given that the
143 reflectance peak is blueshifted for large incident angles (Supplementary Fig. S6).

144

145 Iridoplasts therefore both reflect blue light and show enhanced absorption in the green-red. These
146 *Begonia* species are found in “extreme” shade conditions. The overhead forest canopy absorbs
147 most of the light, such that the intensity reaching the forest floor can be attenuated by up to 10^{-6} -
148 10^{-7} (60-70dB)⁶. Moreover, the spectral distribution of available light is modified: absorption of
149 the ~460nm and ~680nm regions by the canopy above results in a modified spectrum remaining
150 for the understory *Begonia* (Fig. 3a). Enhanced absorption in the green region of the spectrum
151 may therefore be a way to scavenge residual light. The reduction in relative enhancement factor
152 for the blue (460nm) would not be a disadvantage given the very low levels of these wavelengths
153 available, and may even aid photo-protection¹⁴.

154

155 Direct insight into the impact of these modifications on overall photosynthetic efficiency may be
156 gained by using chlorophyll fluorescence imaging of iridoplasts and chloroplasts in the *Begonia*
157 leaf tissue. Chlorophyll fluorescence imaging has become a standard technique to assess
158 photosynthetic parameters in a range of organisms. While it gives no information on how
159 absorption affects the overall efficiency of photosynthesis, this technique provides an estimate of
160 the efficiency (quantum yield) with which absorbed light can be utilized for electron transport
161 and photosynthesis^{21,22}.

162

163 Figure 4 shows that maximum quantum efficiency of PSII photochemistry (F_v/F_m) values from
164 iridoplasts were significantly ($p < 0.05$) and consistently 5-10% higher than those of

165 chloroplasts, both in cross sections and in peels comprising adaxial epidermis and mesophyll
166 (Supplementary Fig. S8). This is surprising given that lower efficiency has been observed in
167 other epidermal chloroplasts²³, and thus it appears that the photonic structure may aid in post-
168 absorptive efficiency. Consistent with previous studies of epidermal chloroplasts however²²,
169 iridoplasts show reduced efficiency in higher light conditions (Supplementary Fig. S9),
170 indicating limitation by electron transport downstream of PSII. Shade-adapted chloroplasts
171 typically show an increase in appressed: non-appressed thylakoids (as seen in iridoplasts) and an
172 associated increase in the amount of PSII antenna complexes²⁴. Interestingly, these adaptations
173 have been shown to reduce quantum yield²⁵ and so it may be that the iridoplast photonic
174 structure compensates for this evolutionary trade-off.

175 The combination of these results suggests that iridoplasts are particularly adapted for low light
176 conditions, where other plants would struggle to grow. Under such conditions the observed
177 reduction in efficiency at higher light levels would not pose a disadvantage to the plant and the
178 potential trade-off of less efficient electron transport would have fewer photodamage
179 implications. While the enhancement of whole plant photosynthesis by the presence of
180 iridoplasts may be marginal, in extreme conditions strong selective pressures would be sufficient
181 to maintain the presence of these structures. This hypothesis is further supported by description
182 of chloroplast structures similar to those found in *Begonia* iridoplasts in a phylogenetically
183 diverse selection of plants from similar deep shade environments, although to our knowledge few
184 of them are described as iridescent and none have yet been modelled as a photonic crystal⁷⁻⁹.
185 This work provides an initial link between physical photonic modification and photosynthetic
186 quantum yield in chloroplasts, but potentially, iridoplasts are just one, clearly visible example of
187 a wide variety of photonic adaptation in light harvesting complexes to be discovered.

188

189

190 **Methods**

191

192 **Electron microscopy.** For transmission electronic microscopy (TEM), leaf tissue was fixed in a
193 2.7% glutaraldehyde solution in 0.1M sodium cacodylate buffer (pH 7.2) at room temperature
194 overnight and post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate for 1.5 hours at
195 room temperature. Specimens were dehydrated in an ethanol series before being embedded in LR
196 white resin (Sigma Aldrich) and 70nm sections taken with a diamond knife. Sections were
197 stained with aqueous uranyl acetate and lead citrate and imaged using a Tecnai T12 microscope
198 (FEI). Representative images are taken from 5 technical repeats for *Begonia grandis x pavonina*.
199 For cryogenic scanning electron microscopy (cryo-SEM) the leaf tissue was plunge-frozen in
200 liquid nitrogen slush and transferred immediately to a Quanta 400 - Scanning Electron
201 Microscope (FEI) for sputter coating with platinum and imaging.

202 **Autofluorescence imaging.** Slides were prepared from dark-adapted leaves either as cross
203 sections or peels containing both adaxial epidermis and palisade mesophyll. Specimens were
204 kept in incubation buffer (50mM KCl, 10mM PIPES, pH 6.8, ²⁶ under a coverslip and further
205 dark adapted for at least 15 mins prior to imaging. Fluorescence images were taken with a
206 Fluorescence Kinetic Microscope controlled with FluorCam7 software (both Photon Systems
207 Instruments). Plastids were selected from fluorescence images using thresholding in FluorCam7
208 and exported data were analysed with Microsoft Excel 2013 and R (version 3.2.3). Values were
209 calculated by averaging data from all plastids in each replicate. Data in Fig. 4b are the means of
210 18 replicates for mesophyll chloroplasts and iridoplasts and 4 replicates for guard cell

211 chloroplasts, from 10 technical repeats. Measurements were discarded if an actinic effect of
212 measuring light was detected, indicated by a non-zero gradient during determination of F0. Data
213 in Supplementary Fig. S9 are the means of at least 6 replicates. Data were analyzed using one-
214 way ANOVA and pairwise t-tests (with Bonferroni correction since the number of comparisons
215 is low). For the data in Fig. 4, one-way ANOVA gave $p = 9.39e-07$. Pairwise t-tests:
216 Iridoplasts:Mesophyll Chloroplasts $p = 0.02$; Guard cell chloroplasts:Mesophyll Chloroplasts $p =$
217 $3.4e-05$; Guard cell chloroplasts:Iridoplasts $p = 5.3e-07$.

218 **Plant material.** *Begonia* plants were grown in glasshouse conditions supplemented with
219 compact fluorescent lighting (Plug and Grow 125W, 6400K, LBS Horticultural Supplies). Plants
220 were grown under benches, with additional shade provided by 50% roof shade netting
221 (Rokolene).

222 **Reflectance measurements.** High resolution imaging and spectra of iridoplasts were performed
223 in vivo on dark-adapted leaves under a coverslip. For characterization we used a custom-made
224 white light epi-illumination microscope. White light lamp illumination (Thorlabs OSL-1) was
225 collimated and focused on the sample with a high NA (1.4) oil immersion lens (Zeiss Plan-
226 Apochromat 100x/1.4 Oil M27). The collected light was then focused onto an optical fiber
227 (Thorlabs M92L01) in a confocal configuration. The optical fiber is connected to an Ocean
228 Optics 2000+ spectrometer for spectral analysis. This configuration ensures the capability to
229 select single iridoplasts in our measurements. A modified configuration of the previous setup
230 allowed Fourier Image Spectroscopy²⁷ over the whole numerical aperture of the objective lens
231 therefore allowing selection of different angles of collection. All reflectance spectra are
232 normalized against a silver mirror (Thorlabs PF10-03-P01) used as the standard for our
233 measurements.

234 **Confocal photoluminescence excitation (PLE).** PLE measurements (also known as Lambda
235 Square Mapping) were performed on a confocal optical microscope Leica SP8X. Using the
236 confocal capabilities we could distinguish PLE signal from iridoplasts and chloroplasts for the
237 same region of the leaf (Supplementary Fig. S3). Values were calculated by averaging the signal
238 from organelles in the same confocal image. For Extended Data Fig 3a the number of samples
239 for iridoplasts was $n = 7$ and $n = 10$ for chloroplasts.

240

241 **Optical model of iridoplasts.** For the calculation of the reflectance spectra of the iridoplast we
242 used an in-house implementation of the Transfer Matrix Method (TMM)²⁸ for unidimensional
243 photonic crystals. We performed a layer by layer calculation defining each layer of the
244 iridoplast. In these simulations each layer needed two parameters as input: refractive index and
245 thickness.

246 Note that unlike in standard calculations in quarter wavelength stacks, the photonic structure
247 under study here presents an ultrastructure in which each high refractive index layer (thylakoid
248 membrane) is less than ten nanometers in thickness. This ultrastructure cannot be approximated
249 by a layer several tens of nanometers thick with a given effective refractive index since
250 variations in thylakoid lumenic thickness would not be accounted for in this simplified model.
251 Therefore, the thickness of each layer is defined with nanometric precision in our calculation.

252 In order to obtain values of those thicknesses, we performed an exhaustive analysis of TEM
253 images to obtain statistics (Extended Data Fig. S4) on the most common values for $M/2$, L and
254 d_s . As described in Fig 2 of the main text, those layers are piled up to form a superstructure
255 where each granum is periodically replicated with a period (A). The granum ultrastructure, that
256 is, the number of thylakoids forming a single granum (N_m), is also obtained from statistical

257 analysis of TEM images (Supplementary Fig. S4). The total grana thickness is then defined as

258 $d_m = N_m(M+L)$.

259 For the refractive indices used in this model we relied upon well-established data in the

260 literature. The low refractive index layers in the iridoplast are stroma and lumen. They are well-

261 known to have an aqueous composition and therefore their refractive index is considered real

262 (non-absorbing) and close to water ($1.3 < n < 1.4$). The exact values however are difficult to

263 obtain²⁹ as well as very variant due to changes in the chemical composition, particularly in the

264 lumen of thylakoids where protein exchange is common¹⁸. In our case we used values

265 $n_L = n_s = 1.35$ as lumen and stroma are known to have very similar values. For the thylakoid

266 membrane, the presence of pigments (especially chlorophyll) will produce a strong material

267 dispersion. The refractive index for the thylakoid membrane ($n_t = n + ik$) is complex (the

268 imaginary part, k , corresponding to absorption) and varies with wavelength. This is partly due to

269 pigments such as chlorophyll (present in both chloroplasts and iridoplasts, absorbing strongly in

270 the blue (~420nm) and red (680nm) which causes a change in the real part of the refractive index

271 at those resonant wavelengths. Refractive index values are shown in Supplementary Fig. S3.

272 In our approach to modelling the iridoplast we defined each layer according to the thickness

273 extracted from ultrastructural analysis. Next we select the refractive index depending on the type

274 of material the layer is formed from. Note that while lumen and stroma will not show material

275 dispersion, the thylakoid membrane refractive index will change strongly with light wavelength.

276 Therefore, in order to define the refractive index of the thylakoid membrane we selected the

277 appropriate complex refractive index for the wavelength of interest as shown in Supplementary

278 Fig. S3. Finally, reflectance was calculated for single wavelengths incident normally to the

279 superstructure and from an aqueous medium with refractive index $n_{in} = 1.33$.

280 Note that the inner chemistry and especially the ultrastructure of chloroplasts and iridoplasts
281 changes to adapt to the light environment²³. Therefore, the reflectance of the iridoplast as
282 calculated with values extracted from TEMs is likely to differ from the actual reflectance
283 measurement given the difficulty of measuring reflectance and TEM of the same iridoplast. The
284 mean values as extracted from TEM (sample size $n = 17$) images (Extended Data Fig. 4) were
285 $M = 6.7 \pm 0.8$ nm, $L = 7.5 \pm 0.8$ nm, $d_s = 94 \pm 16$ nm (sample size $n = 70$). Numbers of grana and
286 thylakoids per granum were: $N = 7 \pm 1$ and $N_m = 3 \pm 1$. When these values were introduced into the
287 model a strongly blue shifted and reduced reflectance was obtained. However, after performing a
288 fine tuning of these parameters we determined that for values $M = 9$ nm, $L = 7.5$ nm and d_s in the
289 range 115-125 nm a very good fit was obtained. The other parameters were $N = 8$ and $N_m = 3$.
290 Finally, for those values the model suggests a period $\lambda = 174.5$ nm with $d_m = 49.5$ nm.

291 **Variance on thicknesses.** The thicknesses of iridoplasts layering predicted by our model are still
292 well within values reported in literature for different membranes and compartments in
293 chloroplasts²⁹ as well as within the errors obtained from our own statistical analysis, except for
294 the case of the thylakoid membrane. The causes of this mismatch are twofold. First, the distances
295 in TEM images are usually underestimated due to shrinkage during the tissue preparation.
296 Therefore the values for L , M and d_s obtained in Supplementary Fig. S4 are likely to be slightly
297 smaller than *in vivo*. On the other hand, cryo-SEM (Fig. 1c) is known to produce more realistic
298 distances²⁹. Interestingly, when an FFT study of cryo-SEM images of a single iridoplast was
299 performed we obtained a period $\lambda \approx 170 \pm 20$ nm (Supplementary Fig S7). This value is very
300 similar to the period suggested by the model, fitting experimental reflectance measurements. The
301 second cause for differences between experimental and modelling values is that the inspection of
302 the thickness of thylakoid membranes and their relation to the actual structure of the organelles

303 *in-vivo* is very challenging. Thickness of L and M is known to change with light adaptation but
304 also the arrangement of grana¹⁰. All our morphological analysis was performed in leaves
305 showing strong iridoplast reflectance after dark adaptation. However, iridoplast reflectance
306 appears to be very sensitive to light conditions. Hence, slight differences in conditions during
307 preparation for electron and optical microscopy might also be responsible for the differences in
308 the values of thicknesses between the model and TEM images. Further work is required on the
309 dynamics of thylakoids in iridoplasts but this is beyond the scope of this study.

310 **Calculation of electric field profiles within an iridoplast under illumination.** For calculation
311 of field profiles we used a commercial Finite Difference Time Domain (FDTD) tool³⁰. We used
312 the same structure and refractive indices used in the TMM implementation. A single wavelength
313 plane wave illumination normal to the multilayer was used as an excitation source and the field
314 was allowed to propagate within the structure for 3ps.

315

316 **Alternative description of the enhanced absorption.** For a simpler structure than the iridoplast,
317 an alternative derivation for the dimensionless enhancement factor γ can be obtained¹⁹:

$$\gamma = f \frac{c/n_l}{v_g} \quad (1)$$

318 where f is a dimensionless parameter representing the overlap in position between the structure
319 of the absorbent material of the photonic crystals and the field distribution. n_l is the real part of
320 the refractive index of absorbent material (bulk) and v_g is the group velocity of the light wave.
321 Therefore, a higher field-grana overlap and lower group velocity will both increase γ as our
322 model demonstrates for iridoplasts due to the presence of the photonic band edge. The total
323 absorptance enhancement parameter (γ_{tot}) mentioned in the caption of Fig. 3 is calculated as:

$$\gamma_{tot} = \int^{PAR} \gamma(\lambda) d\lambda \quad (2)$$

324 Where the Photosynthetically Active Radiation (PAR) range is $400 \text{ nm} < \lambda < 700 \text{ nm}$ and γ the
325 dimensionless enhancement factor at a particular wavelength λ .

326

327

328

329 **Requests for material:** Please contact the corresponding author (heather.whitney@bristol.ac.uk)
330 to request any material. The custom code used for implementation of the Transfer Matrix
331 Method was developed as a Matlab script and is available upon request from Martin Lopez-
332 Garcia (eemlg@bristol.ac.uk).

333

334 **Acknowledgements:** We would like to thank Dr Catherine Kidner, Dr Mark Hughes (Royal
335 Botanic Gardens Edinburgh) and Mr Clive Lundquist (Royal Botanic Gardens Kew) for Begonia
336 species identification and tissue, and Mrs Alanna Kelly for Begonia collection maintenance and
337 horticultural expertise. We thank Stuart Casson and Lucy Sandbach for contributions to pilot
338 studies. We acknowledge the Wolfson Bioimaging Facility (University of Bristol) with thanks to
339 Dominic Alibhai, Judith Mantell and Gini Tilly for assistance with imaging and also thank Dr
340 Silvere Vialet-Chabrand (University of Essex) for assistance with fluorescence microscopy. We
341 also thank Prof Martin J. Cryan, Dr Tom Oliver and Dr. Sara Nunez from the University of
342 Bristol for fruitful discussion on the photonic side of photosynthesis. MJ is funded by a NERC
343 PhD studentship. We are grateful for funding from ERC project number 260920 (to HMW).

344

345

346 **Author Contributions:** M.J., M.L-G., R.O. and H.W. conceived the experiments, M.J., O.P. and
347 M.L-G. carried out iridoplast optical analysis, M.J., M.L-G. and T.S. carried out the
348 photosynthesis experiments, M.J., O.P. and M.L-G. carried out microscopy, M.L-G. and R.O.
349 designed and ran optical models, M.J., M.L-G., R.O. and H.W. wrote the manuscript, which all
350 authors commented on.

352 **References**

- 353 1. Bermel, P., Luo, C., Zeng, L., Kimerling, L. C. & Joannopoulos, J. D. Improving thin-film
354 crystalline silicon solar cell efficiencies with photonic crystals. *Opt. Express* **15**, 16986–
355 17000 (2007).
- 356 2. Mihi, A. & Miguez, H. Origin of light-harvesting enhancement in colloidal-photonic-
357 crystal-based dye-sensitized solar cells. *J. Phys. Chem. B* **109**, 15968–15976 (2005).
- 358 3. Holt, A. L., Vahidinia, S., Gagnon, Y. L., Morse, D. E. & Sweeney, A. M. Photosymbiotic
359 giant clams are transformers of solar flux. *J. R. Soc. Interface* **11**, 20140678 (2014).
- 360 4. Glover, B. J. & Whitney, H. M. Structural colour and iridescence in plants: the poorly
361 studied relations of pigment colour. *Ann. Bot.* **105**, 505–511 (2010).
- 362 5. Gould, K. S. & Lee, D. W. Physical and ultrastructural basis of blue leaf iridescence in
363 four Malaysian understory plants. *Am. J. Bot.* **83**, 45–50 (1996).
- 364 6. Endler, J. A. The color of light in forests and its implications. *Ecol. Monogr.* **63**, 1–27
365 (1993).
- 366 7. Sheue, C., Sarafis, V. & Kiew, R. Bizonoplast, a unique chloroplast in the epidermal cells
367 of microphylls in the shade plant *Selaginella erythropus* (Selaginellaceae). *Am. J. Bot.* **94**,
368 1922–1929 (2007).
- 369 8. Graham, R. M., Lee, D. W. & Norstog, K. Physical and ultrastructural basis of blue leaf
370 iridescence in two neotropical ferns. *Am. J. Bot.* **80**, 198–203 (1993).
- 371 9. Nasrulhaq-Boyce, A. & Duckett, J. Dimorphic epidermal cell chloroplasts in the
372 mesophyll-less leaves of an extreme-shade tropical fern, *Teratophyllum rotundifoliatum*
373 (R. Bonap.) Holtt.: a light and electron microscope study. *New Phytol.* **119**, 433–444
374 (1991).
- 375 10. Kirchoff, H. Architectural switches in plant thylakoid membranes. *Photosynth. Res.* **116**,
376 481–487 (2013).
- 377 11. Pribil, M., Labs, M. & Leister, D. Structure and dynamics of thylakoids in land plants. *J.*
378 *Exp. Bot.* **65**, 1955–1972 (2014).
- 379 12. Vukusic, P. & Sambles, J. R. Photonic structures in biology. *Nature* **424**, 852–855 (2003).
- 380 13. Sun, J., Bhushan, B. & Tong, J. Structural coloration in nature. *RSC Adv.* **3**, 14862–14889
381 (2013).
- 382 14. Thomas, K. R., Kolle, M., Whitney, H. M., Glover, B. J. & Steiner, U. Function of blue
383 iridescence in tropical understory plants. *J. R. Soc. Interface* **7**, 1699–1707 (2010).
- 384 15. Lee, D. W. *Nature's Palette: the Science of Plant Colour*. (The University of Chicago
385 Press, 2007).
- 386 16. Gebushuber, C. I. & Lee, D. W. in *Springer Encycl. Nanotechnol.* (Bhushan, B.) 1790–
387 1803 (Springer Netherlands, 2012). doi:10.1007/978-90-481-9751-4
- 388 17. Pribil, M., Labs, M. & Leister, D. Structure and dynamics of thylakoids in land plants. *J.*
389 *Exp. Bot.* **65**, 1955–1972 (2014).

- 390 18. Kirchhoff, H. Structural changes of the thylakoid membrane network induced by high
391 light stress in plant chloroplasts. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **369**, 20130225
392 (2014).
- 393 19. Pedersen, J., Xiao, S. & Mortensen, N. A. Slow-light enhanced absorption for bio-
394 chemical sensing applications: Potential of low-contrast lossy materials. *J. Eur. Opt. Soc.*
395 **3**, (2008).
- 396 20. Nevo, R., Charuvi, D., Tsabari, O. & Reich, Z. Composition, architecture and dynamics of
397 the photosynthetic apparatus in higher plants. *Plant J.* **70**, 157–176 (2012).
- 398 21. Murchie, E. H. & Lawson, T. Chlorophyll fluorescence analysis: a guide to good practice
399 and understanding some new applications. *J. Exp. Bot.* **64**, 3983–3998 (2013).
- 400 22. Lawson, T., Oxborough, K., Morison, J. I. L. & Baker, N. R. Responses of photosynthetic
401 electron transport in stomatal guard cells and mesophyll cells in intact leaves to light,
402 CO₂, and humidity. *Plant Physiol.* **128**, 52–62 (2002).
- 403 23. Lawson, T., Oxborough, K., Morison, J. I. L. & Baker, N. R. The responses of guard and
404 mesophyll cell photosynthesis to CO₂, O₂, light, and water stress in a range of species are
405 similar. *J. Exp. Bot.* **54**, 1743–1752 (2003).
- 406 24. Kouřil, R., Wientjes, E., Bultema, J. B., Croce, R. & Boekema, E. J. High-light vs. low-
407 light: Effect of light acclimation on photosystem II composition and organization in
408 *Arabidopsis thaliana*. *Biochim. Biophys. Acta - Bioenerg.* **1827**, 411–419 (2013).
- 409 25. Wientjes, E., Van Amerongen, H. & Croce, R. Quantum yield of charge separation in
410 photosystem II: Functional effect of changes in the antenna size upon light acclimation. *J.*
411 *Phys. Chem. B* **117**, 11200–11208 (2013).
- 412 26. Lawson, T., Oxborough, K., Morison, J. I. L. & Baker, N. R. Responses of photosynthetic
413 electron transport in stomatal guard cells and mesophyll cells in intact leaves to light,
414 CO₂, and humidity. *Plant Physiol.* **128**, 52–62 (2002).
- 415 27. Lopez-Garcia, M. *et al.* Enhancement and directionality of spontaneous emission in hybrid
416 self-assembled photonic-plasmonic crystals. *Small* **6**, 1757–1761 (2010).
- 417 28. Yeh, P., Yariv, A., Hong, C. & Introduction, I. Electromagnetic propagation in periodic
418 stratified media. I. General theory. *J. Opt. Soc. Am.* **91125**, 423–438 (1977).
- 419 29. Kirchhoff, H. *et al.* Dynamic control of protein diffusion within the granal thylakoid
420 lumen. *Proc. Natl. Acad. Sci.* **108**, 20248–20253 (2011).
- 421 30. Lumerical Solutions, Inc. <http://www.lumerical.com/tcad-products/fdtd/>. Version 8.15.736.
422 (2015).
- 423

Figure 1 | Blue leaf iridescence and iridoplasts in *Begonia*. **a**, Photograph of a leaf of *Begonia grandis x pavonina* (GxP). **b**, microscopy image showing iridoplasts in an epidermal cell under bright field epi-illumination. **c**, cryo-SEM image of a single iridoplast in *Begonia GxP*. **d**, typical reflectance spectrum measured at normal incidence for a single iridoplast (inset). Colour bar indicates real colors.

Figure 2 | Optical properties and modelling of iridoplast structure. **a**, TEM image of iridoplast (scale bar 1 μ m) and inset of ultrastructure. Sketches show the parameters used in the optical model to define the photonic structure. **b**, experimental reflectance spectra normalized to maximum (absolute values available in Supplementary Fig.7) for two GxP iridoplasts. Solid and dotted red lines are calculated reflectance for $d_s = 125$ nm and $d_s = 115$ nm respectively with $N = 8$, $N_m = 3$, $M = 9$ nm and $L = 7.5$ nm. **c**, iridoplast reflectance spectra as a function of the spacing between grana (d_s). Dashed line is $d_s = 125$ nm

Figure 3 | Enhanced absorption at reflectance sideband. **a**, calculation of γ at normal incidence for the iridoplasts in Fig 2b. Shadow area shows integration area for the calculation of $\gamma_{tot} = 5.6$ (7.6) nm with $d_s = 125$ (115) nm (see Methods). Bottom figure shows PAR spectra (q_{norm} not at scale) under direct sun (yellow) and forest canopy shade (green)⁶. **b**, Electric field intensity within the iridoplast for wavelengths of interest shown as dashed lines in a) and $d_s = 125$ nm. Light (dark) grey regions represent stroma (grana) and y position within the structure. **c**, γ as a function of incident angle (θ) and wavelength for $d_s = 125$ nm and unpolarised incident light.

Figure 4 | Chlorophyll fluorescence images and quantum yield of *Begonia* plastids. **a**, chlorophyll fluorescence images of mesophyll chloroplasts (top) and iridoplasts (bottom). **b**, Maximum quantum yield of photosystem II (Fv/Fm) for guard cell chloroplasts, iridoplasts and mesophyll chloroplasts. Error bar shows standard deviation of the measurements.