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STRUCTURAL FRUIT COLORATION IN *DELARBREA MICHIEANA* (ARALIACEAE)

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The brilliant blue fruit color of *Delarbrea michieana* (F. Muell.) F. Muell. (Araliaceae), a Queensland understory rain forest tree, is caused by iridisomes (structures) in the epidermal cells that are produced beneath the cell wall and probably outside of the cytoplasm. Layers within these iridisomes are of such a thickness that they interfere constructively with light at 420–440 nm and produce the color. Such color production may aid in attracting mammals and large frugivorous birds (which may disperse the fruits) and may also allow ripe fruits to continue photosynthetic carbon assimilation.

Keywords: Araliaceae, *Delarbrea*, dispersal, fruit color, interference, rain forest, tropical.

Introduction

The fruits of *Delarbrea michieana* (F. Muell.) F. Muell. (Araliaceae), a small understory tree of north Queensland rain forests, are a brilliant iridescent blue. The color of nearly all fleshy fruits is the result of pigmentation. The selective absorption of light scattered through the epidermis and cortex produces the yellows, reds, blues, and violet-blacks of fruits; these colors are presumably signals to aid in dispersal of these fruits. Blue color, which is present in ca. 5%–7% of fleshy fruits (Wheelright and Janson 1985), is caused by anthocyanins, which are modified by metallic cations or by copigmentation. When crushed, such fruits yield a juice that is colored pink-red by these pigments. However, the fruits of numerous taxa within *Elaeocarpus* (Elaeocarpaceae), such as *Elaeocarpus angustifolius* Blume, produce brilliant blue coloration from constructive interference (Corner 1988), which is caused by structures (iridisomes) within the epidermis (Lee 1991). Although such a basis for fruit coloration may be exceedingly rare, in this paper we describe the structural basis for iridescent blue fruit color in *D. michieana* and speculate on its phylogenetic and ecological significance.

Material and Methods

Delarbrea michieana is endemic to the rain forests of the wet tropical region of Queensland (between Cooktown and Tully), extending over a latitudinal range between 15°30′ and 18°00′S at altitudes between sea level and 1100 m. We collected ripe fruits from individual trees in a 25-yr-old secondary forest located 3 km east of Atherton. The elliptical fruits of these trees, which are 19–24 mm long × 11–15 mm in diameter, were obtained from trees in the Curtain Fig Forest—State Forest 452 (Atherton Tablelands; voucher, K. Sanderson 1008, QRS). We also examined fruits of several closely related species within the Araliaceae, including *Delarbrea montana* Vieill. ex R. Viguier (Lowry 4759), *Delarbrea harmsii* R. Viguier (Lowry 4732), *Delarbrea paradoxa* Vieill. (Lowry 4791), *Delarbrea michieana* (Plunkett 1502), and *Mackinlaya macrosciadea* (F. Muell.) F. Muell. (Plunkett 1549), preserved in FAA (obtained from A. Oskolski).

We determined the peak wavelengths of the structural blue coloration of five ripe fruits of *D. michieana* by measuring diffuse reflectance with a Li-Cor 1800 spectroradiometer (LiCor Instruments, Lincoln, Nebr.) with integrating sphere attachment (Lee 1991; Gould and Lee 1996).

For examination by transmission electron microscopy (TEM), we fixed 1-mm^3 cubes of tissue in half-strength Karnovsky's solution (Karnovsky 1965) and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer. After dehydration in a 50%–100% series of ethanol, we infiltrated and embedded specimens in Spurr resin. Thin sections cut with a DDK diamond ultramicrotomy knife on a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate and lead citrate and photographed in a Philipps EM300 microscope. For photomicrography, we cut 1-μm sections with a DDK diamond histoknife on a Reichert-Jung 2050 microtome and stained them with 0.1% toluidine blue in 1% sodium borate. We also stained for cellulose with calcofluor white M2R (Fischer et al. 1985). We examined fruit tissue from the other taxa by light microscopy of hand sections stained with 0.1% toluidine blue. We analyzed photographic enlargements of three fruits, making transects through four iridisomes of each section and measuring distances with a digital caliper.

Results and Discussion

Predictions of Filter Thickness

Among animals, structural coloration is caused by constructive interference, diffraction, and Tyndall scattering by small particles (Fox 1976). Coloration by pigmentation in *Delarbrea michieana* can be ruled out because no red or blue pigment was extractable, and the blue layer did not produce color when light was transmitted through it. Diffraction cannot account

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for the color because of the relative independence of color production with incidence angle. Tyndall scattering requires a dark-pigmented background and produces progressively greater reflectance at shorter wavelengths, which was not seen in these fruits.

Interference coloration requires the presence of filters/layers of an appropriate thickness and a different refractive index η from the surrounding medium. In plants, a η of 1.45, that of hydrated cellulose and cell membranes, would be the logical index value for such a filter, higher than an aqueous medium of 1.33 (Gould and Lee 1996). Filter thickness can be predicted using the following standard formula for thin-film interference (Jenkins and White 1957):

$$\text{thickness } (t) = \lambda/4\eta\cos\theta.$$

In this equation, λ is the peak wavelength of constructive interference (and color production) in nanometers and θ is the refracted angle of light in the filter (1 for an angle of incidence of 0° for vertical illumination). The value 4 converts to value 2 when the filter has a lower η than surrounding layers and phase reversal occurs. Based on a broad peak of diffuse reflectance of 420–440 nm (fig. 1), we have predicted the possible thicknesses of such a filter in the fruit epidermis (table 1). Verification of such a structure (ca. 70–150 nm thickness) requires fine structure imaging by TEM.

Ultrastructural Basis of Constructive Interference

Epidermal cells of *D. michieana* fruits, where the iridescent blue color originates, are columnar in shape (fig. 2*A*). The external end of these cells, beneath the wall, contains a clear region that became medium blue after staining with toluidine blue. This region, the likely origin of color production, revealed a complex and multilayered structure, based on TEM (fig. 2*B*–2*F*). We call such a structure an "iridisome" (Lee 1991). The iridisome consists of numerous layers with electron-translucent "bubble-like" regions that are bounded by electron-opaque "wall-like" regions. These layers vary in thickness in the entire structure, being thicker in its center. For measurements to test predictions of interference, we used the multiple layers near the outer margin (table 1). This region would absorb the most light and would best account for color production. The layer in this structure that is most likely to account for constructive interference is the electron-translucent bubble-like region, with a measured thickness of 75 nm. This is within the predicted thickness of 72–76 nm for a layer with a reduced η relative to surrounding layers (table 1). Paradermal sections (fig. 2*E*) indicate that these layers form plates, with no evidence of a different vertical structure, as observed in *Elaeocarpus angustifolius* (Lee 1991).

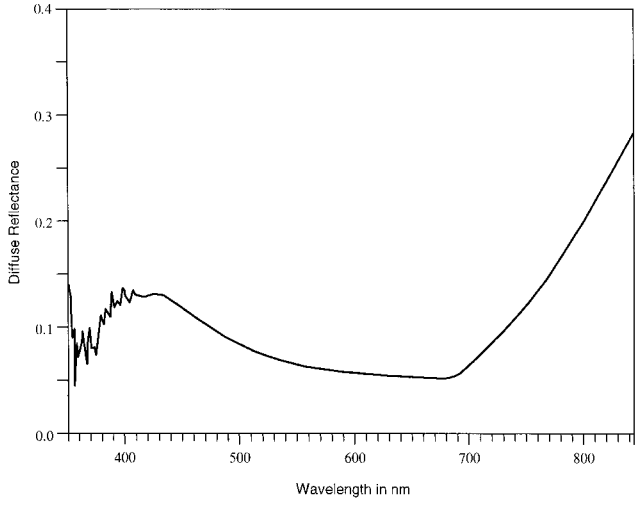

Fig. 1 Representative scan of diffuse reflectance of fruit of *Delarbrea michieana*.

Table 1

Predicted Filter Thicknesses, Based on Diffuse Reflectance of 420–440 nm and Measured Thicknesses of Structures in the Epidermal Cells of *Delarbrea michieana*

	$2\eta t$ (nm)	$4\eta t$ (nm)
Theoretical	145–152	**72–76**
Measured:		
Electron opaque layer ("wall")	27 ± 5	...
Electron translucent layer ("bubble")	**75 ± 6**	...
Both layers	101 ± 12	...

Note. We have based these predictions on an equation of $\lambda = 4\eta t$ (with no phase change) and $\lambda = 2\eta t$ (with phase change). Numbers highlighted in boldface indicate the most probable match for the theoretical and measured structure for the color production.

The iridisomes are produced within the adaxial cell wall (fig. 2*D*) and adjacent to the cytoplasm (fig. 2*C*), although we could not observe a clear plasmalemma between the cytoplasm and the iridisome. Apparently the structure is secreted by the cytoplasm during the ripening of the fruit in a manner analogous to that observed in *E. angustifolius,* although the structures of the iridisomes of these two distantly related taxa appear to be quite different from each other (Lee 1991). The iridisomes in *E. angustifolius* have a pronounced linear vertical structure and a "spaghetti-like" appearance in transverse section. Given the moderate affinity of the entire structure in *D. michieana* for toluidine blue and its strong binding of calcofluor white, the molecular basis of the iridisome is primarily cellulose, which is similar to that for *E. angustifolius*.

Phylogenetic and Ecological Implications

Structural fruit coloration in *D. michieana* appears to be unique within the genus and probably also within the family. The only other known taxa with iridescent fruit are members of the Elaeocarpaceae, which is phylogenetically distant, and these characters are clearly homoplasious. We examined fruit structure in other taxa within *Delarbrea* and in a related genus of blue-gray fruited trees in the Araliaceae (*Mackinlaya*). The fruits of all of these taxa produce fruit with blue-purple color, but these fruits do not display the metallic intensity of *D. michieana* (Lowry 1986). In light microscope observations, the epidermal cells of these taxa were cuboidal in shape and devoid

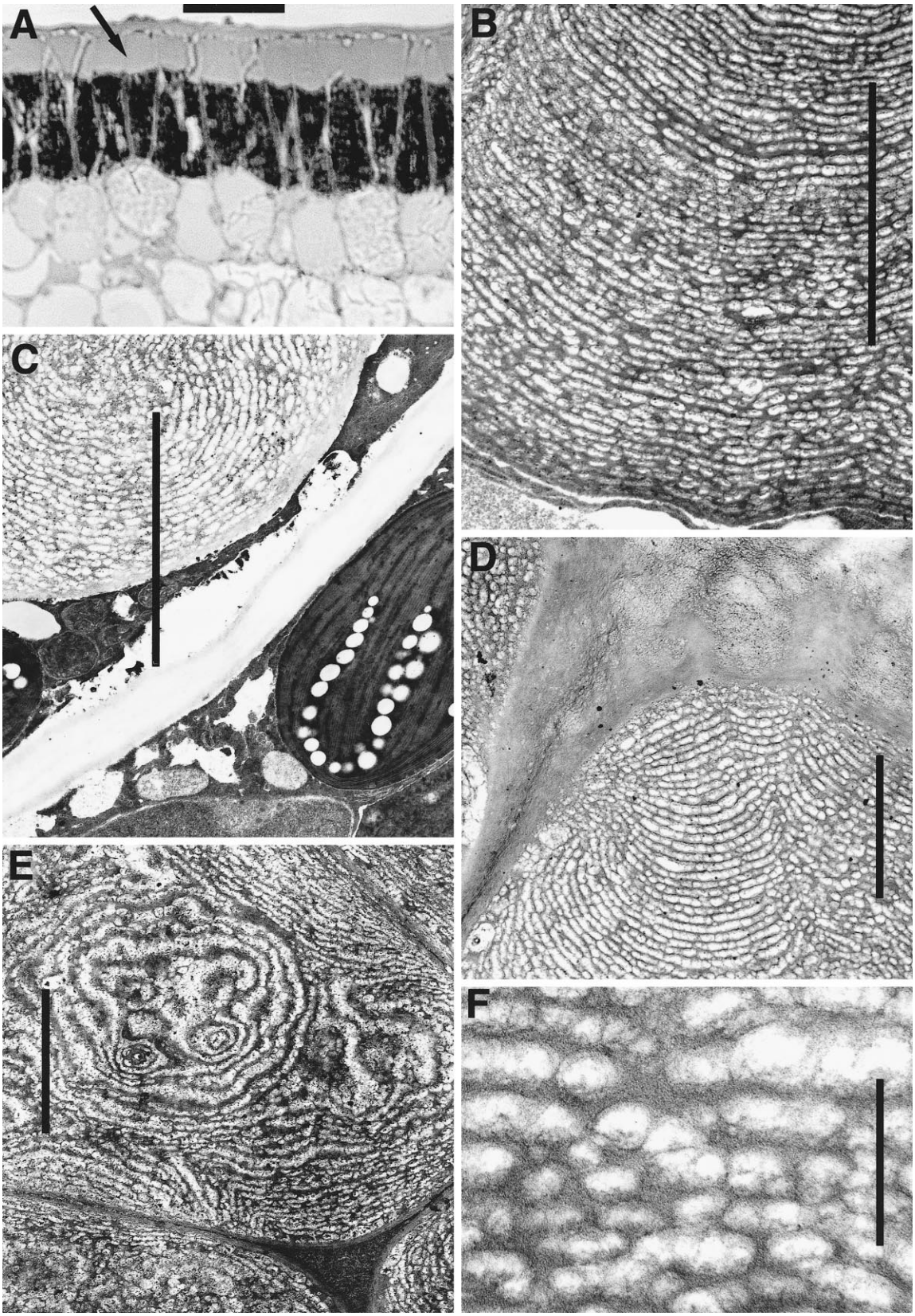

Fig. 2 Light (*A*) and transmission electron (*B–F*) micrographs of fruits of *Delarbrea michieana*. *A*, Epidermis and cortex of fruit. Arrow points to an iridisome. Bar = 100 μm. *B*, Detail of iridisome toward inner wall, showing relationship to cytoplasm. *C*, Paradermal section through iridisome near outer cell wall, showing adjacent cytoplasm with functioning chloroplasts. *D*, Detail of iridisome near the outer cell wall, showing relationship to cell wall. *E*, Paradermal section through middle of iridisome, revealing the platelike structure of the layers. *B–E*, Bars = 2 μm. *F*, Detail of iridisome in transverse section, showing electron opaque "wall-like" layers and electron translucent "bubble-like" layers. Bar = 500 nm.

of the clear structure immediately beneath the external cell wall (the iridisome of *D. michieana*).

The distinctness of *D. michieana* (compared with other taxa in the genus) has possible phylogenetic implications. *Delarbrea* comprises six species, centered in New Caledonia (Lowry 1986); *D. michieana* is the only Australian member of the genus, which is probably a remnant of the mid-Eocene Australasian flora that has survived both in Australia and New Caledonia (White 1998). Climatic changes reduced the area of rain forest and removed many groups from Australia (Raven 1980). A subsequent Miocene collision with the Sunda arc (Barlow 1981) could have permitted the arrival of *Delarbrea*, as could the sea-level drops associated with recent glaciation (White 1998). Molecular sequence data (G. Plunkett, unpublished data) indicate that *D. michieana* is nested within a clade comprising its congeners and is perhaps most closely related to *D. paradoxa*. These results indicate that the presence of *D. michieana* in Australia is more likely the result of more recent dispersal because, if the distribution of *D. michieana* were relictual, it would probably occupy a basal position within the clade. Thus, the structural fruit coloration in *D. michieana* is apparently an autapomorphy, having evolved after dispersal to Australia.

The ecological significance of fruit color is problematic. Lee (1991) argued that iridescent color could provide a brilliant and more persistent mode of coloration, thereby promoting dispersal as well as fruit wall transparency that would allow for photosynthetic carbon fixation in ripe fruits. Indeed, the cortex of the ripe fruits of *D. michieana* was green, and the inner cytoplasm of the epidermal cells contained chloroplasts (fig. 2*C*), indicating that photosynthesis occurred within them. Fruits of *D. michieana* are consumed and dispersed primarily by medium- to large-sized birds (A. K. Irvine, personal observations). The southern cassowary (*Casuarius casuarius*), fruit pigeons, the metallic starling (*Aplonis metallica*), and the satin bowerbird (*Ptilorhynchus violaceus*) are the main dispersers that are active during the day. Bennett's tree kangaroo (*Dendrolagus bennettianus*) and the Lumholz tree kangaroo (*Dendrolagus lumholtzi*) are the main known mammal dispersers; these species are mainly nocturnal. Fruits of *E. angustifolius* are also dispersed by a variety of birds and mammals (Willson et al. 1989). At least two other species in this region may also produce iridescent blue fruits (Cooper and Cooper 1994): *Athertonia diversifolia* (C. White) L. Johnson and B. Briggs (Proteaceae) and *Cerbera inflata* S. T. Blake (Apocynaceae).

Thus, the fruit of *D. michieana* attracts both arboreal and terrestrial bird and mammal dispersers. The delayed ripening of the mesocarp (of both this and perhaps other taxa in the region) may be an attraction to animals of particular importance in this rain forest ecosystem. It is likely that understanding related to the selective advantage of fruit iridescence may be found in a closer study of the relationship between these fruits and the particular animals that disperse them.

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