

# BULLETIN OF THE ALLYN MUSEUM

3701 Bayshore Rd.  
Sarasota, Florida 33580

Published By  
The Florida State Museum  
University of Florida  
Gainesville, Florida 32611

Number...75

15 October 1982

---

---

## MICROSTRUCTURE OF BLUE/GREEN AND YELLOW PIGMENTED WING MEMBRANES IN LEPIDOPTERA With Remarks Concerning the Function of Pterobilins 1. Genus *Graphium*

A. C. ALLYN  
Allyn Museum of Entomology

MIRIAM ROTHSCHILD  
Ashton Wold, Peterborough

D. S. SMITH  
Department of Zoology, Oxford University,  
and the Hope Entomological Collections

### INTRODUCTION

Relatively few studies have been made on the structure of the coloured wing membranes which, if they are not obscured by the presence of overlying scales, can play an important role in the visual patterns of butterflies.

We have here concentrated on the modifications associated with such exposed coloured membranes in selected species of the genus *Graphium*. In this group of swallowtails there are large areas of both hind- and forewings which are bright blue and blue-green in colour, occasionally yellow and in one instance mauve and green - but are devoid of the usual type of wing-scale covering and thus afford an excellent opportunity for examining the microstructure and pigmentation of the wing membranes themselves.

Studies of other genera with similar modified structures will be the subject of a continuing series of publications.

### MATERIALS AND METHODS

Reflectance and absorbance were measured on a Beckman (DB-GT) grating spectrophotometer using a 10° specular reflectance accessory and a beam attenuation of 92%. The samples were charted using a Beckman 10-inch recorder in a continuous readout from 190 nm to 700 nm.

Standard light microscopy was also used to recognize and record specific colours produced by scales and membranes prior to coating for study by scanning electron microscopy. Microscopy using an ultraviolet light source (350 nm peak) was used to aid in visual differentiation of pigment location.

A JSM-U3 scanning electron microscope was used to examine the surface micromorphology of the samples. The samples were prepared by coating (ca. 50Å) with 60/40 gold palladium alloy in a Varian VE10 vacuum coater.

For TEM study samples were fixed in 2.5% glutaraldehyde (in 0.05M cacodylate buffer, pH 7.4) for one hour, washed and then placed in 1% osmium tetroxide (same buffer) for one hour. They were then dehydrated in an ethanol series, and embedded in Araldite via propylene oxide. They were sectioned with a diamond knife (ca. 600-900Å), stained with uranyl acetate and lead citrate and examined in a Philips EM 200 transmission electron microscope.

The dual wing membranes were separated using a Circon microsurgical probe after affixing the sample to an SEM holder.

Dried specimens of *Graphium* were selected for this study, including sufficient samples to indicate degrees of individual variation within certain species. Specimens of *G. sarpedon* and *G. antheus* were examined alive.

## RESULTS

### *Graphium sarpedon sarpedon* (Linnaeus)

#### General pattern

This handsome swallowtail, which is found throughout the Indo-Australasian region, is dark brownish black on the dorsal surface with a single broad median band of matte blue colour - the exposed pigmented wing membranes - extending into the hind wings. The latter are further embellished by five blue lunules arranged round the margin. The underside is lighter brown with a few patches of red and darker brown on the hind wings. The median band appears more greenish in tone and less matte than if seen from above. On the dorsal surface the blue band is broken up into discrete patches of colour by the wing veins which cross it at intervals. These veins are usually clothed in light scales in the nominate species, but are black in some examples and always so in *G. s. nipponum*. In the anterior portion of the wing there is a tendency for the blue colour to change to blue/green. This is due to the blue pigment in the exposed membrane being less dense in this region and the unidentified pale yellow pigment also present in the membrane is then allowed to mix in the eye of the beholder to produce the well-known blue/green colour so characteristic of the genus. The pale amber tint of the transparent ventral scales possibly adds to the greenish effect. This is especially noticeable when the membranes are seen from the underside of the wings.

All the blue or blue/green areas of the membrane are covered with minute grainy papillae (figures 1-8). Piliform scales replace the usual type (figures 9-23) in the region of the median band on the dorsal surface, which is covered on the ventral surface by transparent, very pale, amber-coloured scales. The distal portion of these scales may be white or opaque.

The wing veins, when the butterfly is alive and if not covered by scales (or if rubbed clear of them), range from pale green in shade to vivid emerald green. The subcostal vein Sc, owing to the thickness of the cuticle, is golden brown when viewed with the light microscope. The haemolymph is bright green when it oozes from a severed vein; the presence of carotenoids (which are scarce in *G. sarpedon*, see Discussion below) adds to the particular yellowish-green tone of this fluid.

The first portion of the median blue band of the hind wings, which is hidden by the basal edges of the forewing, is white, not blue, and although the piliform scales are present, there are no papillae in this area, but the membrane is highly rugose and somewhat reflective. The pale, amber-coloured scales on the ventral surface are present, but they do not affect the overall whitening of this area.

### Modified scales and sockets

In the blue areas of the median band on the upper wing surface lamellate scales are lacking and are replaced by rows of piliform scales or setae. The ventral surface of the blue-green areas of the median band is covered with a single layer of transparent, lamellate scales, rounded at the apex, which are faintly yellowish brown or amber in tone, which also allows the blue/green pigment in the membranes to be seen from outside.

The rows of piliform setae are spaced approximately 0.04 mm apart, and varying in length from 0.08 to 0.1 mm with a diameter of approximately 4  $\mu\text{m}$  and thus do not obscure the pigmented membrane from view. The piliform scales are much longer in parts of the median band of the hindwings than those of the forewings.

At transition areas (figure 24) between the blue/green patches and black/brown areas of the wings, which are usually abrupt, some aberrant scales may be found. These can consist of dark brown club-shaped or more conventionally shaped, brown, lamellate scales, but with drastically reduced lateral flanges, or even the usual setal type, only pigmented dark brown instead of white. In these transition areas, there may also be some aberrant scale sockets of the brown scales, in which papillae are present.

The transparent, amber-coloured scales on the ventral surface are most easily seen when the light source is at a low angle to the membrane surface. They opalesce throughout the visible spectrum, but only with the light source originating in a plane perpendicular to the ridge lines.

Scales attached to, or lying alongside, wing veins are often varied in shape and size. Some are narrow, attenuated and sparse, thus enabling the green or yellow veins to participate in the colour pattern of the wings, while at the other extreme the veins are densely clothed and completely concealed by the conventional, broad, lamellate form of scale.

The sockets of the piliform scales are usually erect and larger than those of the lamellate scales. Viewed through the light microscope, they appear like rows of gravelly blue cones. The intermembranal socket base appears as a flat, circular disc, devoid of signs of an internal bulbous covering (figures 27 and 28). The sockets of both the piliform scales and the transparent scales on the ventral surface terminate in the matrix between membranes without being encapsulated by a bulb-like vagination commonly found in areas lacking heavily pigmented membranes (28a). The sockets of the brown pigmented, lamellate scales which cover the adjoining areas of the wings are of the ordinary type, semi-erect, and the membrane round them is consequently not drawn up into such large folds.

### The papillae

The dorsal surface of the wing membranes in the blue/green region of the median band is covered with minute lens-like structures or papillae, while the ventral surface lacks these projections and displays only lightly roughened surface irregularities (figure 1). In *G. sarpedon* they are colourless, irregular in shape, but roughly rounded at the apex (figure 1a) with sides nearly parallel, averaging in height ca. 0.47  $\mu\text{m}$  and ca. 0.25  $\mu\text{m}$  in width. These papillae are located immediately above the densely pigmented areas of the median band in which the membrane can be seen from without. They are lacking in the pale, greenish areas of the membrane which are concealed by a covering of pigmented brown, black, or red lamellate scales. The blue colour, together with the nodules, are densest around the sockets of the piliform scales of the dorsal surface of the median band. This is well shown in figure 24, where the termination of the papillae and the sockets of the lamellate, brown scales are aligned. No pattern could be discerned in the distribution of the scattered clusters.

### Wing lamellae and matrix

In Figures 29 and 31 the papillae are shown where they overlie a number of distinguishable lamellae (29a). In *G. sarpedon* the periodicity is ca. 40 nm. This lamellate cuticle is always thicker on the dorsal than on the ventral surface, e.g. ca. 1  $\mu\text{m}$  and 0.5

$\mu\text{m}$  respectively (figure 32). Beneath these parallel series of laminations is a region of "spongy" cuticle (29b) which shows the "C-shaped" or parabolic pattern characteristic of cuticle in which the microfibrils are laid down in a helicoidal manner. There are usually 3-4 layers of this cuticle in the dorsal part of the wing and most often poorly defined layers in the lower half.

The central part of the wing membrane is filled with either an amorphous or granular matrix (presumably the dried remnants of the cytoplasm). Within this matrix occur the pigment granules (29c). In our TEM preparations no structural details are visible and the contents of these granules appear to have been lost during preparation. The clear lacunae of various circular and oblong profiles, seen in TEM photos (figures 29-32), are the sites of the pigment granules. The variation in cuticular form and thickness of the wing membrane is determined by the epidermal cells of the pupal wing epithelia, and it is clear from specimens examined by thin sectioning that the bulk of the intracellular pigment granules are restricted during development to the more extensive regions of cytoplasm flanking the surface projections.

The differences in the upper and lower membranes, as well as the location of the intermembrane pigment, may also be noted in SEM figures 1, 8, 25-28. Distinguishing papillae (8a) mark a fracture in the dorsal wing membrane, through which the upper surface of the lower wing membrane (8b) can be seen. Note the "detritus" (8b) on the surface, which is some granular, interlamellar matrix containing the pigment material, which has pulled away from the upper membrane. When these sheets of tissue were originally formed, they were separate, but in the dried condition of the wing it is rather difficult to get any separation of the wing membranes: it is noteworthy, however, how stable these dried structures are, particularly the internal matrix. This matrix and its included pigment survived fixation, extraction and imbedding without any colour change. The pigment only became mobile upon treatment with 0.1 N KOH.

### Pigment granules

Individual pigment granules appear to be oblate spheroids about 0.5 microns in the long dimension and perhaps 0.25 microns in the short dimension. They are thus comparable in size to those bodies deposited in the scales of most Pierids. They are present in small numbers throughout the wings, but are concentrated round the "hillocks" or anticlinal elevations at the base of the setae of the median band. Compare the concentration of pigment granules in figures 29, 31 and 32 near setal sockets with those in figure 30, showing an area which is not near a socket. This figure also demonstrates the thickening dorsal cuticle and lamellations, and the increased central matrix containing pigments.

### Colouration

The blue/green colouration of the wing membranes and wing veins of *Graphium sarpedon* is due to the presence of pterobilins (see Discussion and figure 37). These bile pigments, when present in the haemolymph, mix physically with carotenoids to produce varying shades of green. They are most concentrated in the membranes in the basal patch (of the blue median band) and along the anal vein (2A) and here take on a mauve tint, suggesting the presence of phorcabilin - a metabolite of pterobilin. Where these pigments are located in scale sockets, or between sockets, in those parts of the wing membrane covered by pigmented scales, they are pale green, rather than blue. Blue colouration only occurs in regions in which the papillate surface is found, although these nodules are not limited to such areas and occur in the anterior portion of the median band, which is distinctly greener than blue. There is, however, considerable variation in colour density between specimens of the same brood.

In some subspecies, such as *G. s. anthedon* Felder, the whole median band of both fore- and hind-wings is more intensely blue than usual. In others the lunules of the hindwing are larger than in *s. sarpedon* and intensely blue, associated with a narrow median band, while again in others the median band is wide and confluent, paler in tone and the lunules greatly reduced.

### **Graphium agamemnon agamemnon (Linnaeus)**

The dorsal surface of the yellow/green membrane in this species is structurally identical with the blue/green membrane of *G. sarpedon*. (figure 2). The rows of piliform scales are spaced approximately .04 mm apart. They are colourless and vary in length from .08 to .10 mm, with a diameter of approximately 4  $\mu$ m (figures 9 and 10) similar to that of *G. sarpedon*. On the ventral surface substantial differences occur. The cover scales of the greenish yellow pigmented areas are variably dentate and have large windows. They opalesce only in the yellow to red portion of the visible spectrum and this opalescence is limited to the area associated with the ridge lines. The proximal half of the ventral greenish-yellow membrane areas contains papillae identical with those of the dorsal surface, while the distal half of the area is smooth.

The wing membranes are more or less symmetrical as in *G. sarpedon* (figures 33 and 34) consisting of the outer lamellate (33a) and non-lamellate (33b) cuticle. The central zone of cellular remnants (33c) contains the pigment granules. These granules are less well defined than in *sarpedon* and seem to have a microcrystalline or spicular component.

### **Graphium weiskei (Ribbe)**

This unusually beautiful species has the two basal blocks of the median band suffused with a purplish pink and the same colour is repeated in the two lunules in the hind-wing above the tails. The structure of the dorsal surface of the purple pigmented area is similar to that of the blue/green wing membranes of *G. s. sarpedon* and *G. a. agamemnon*. The piliform scales, however, are longer, being approximately .13 mm in length (figure 23). Along the surface and sides of the bright green nervules the lateral scales are often reduced to black setae.

The papillae of the dorsal surface of the wing membrane appear to be significantly longer and more sharply tapered than those of the other species. The apices are nearly pointed, not knoblike as they usually are in the other species examined, with non-parallel sides and maybe 3-4 times as high as they are wide. The irregular shape is matched, as in *G. sarpedon*, by an irregular distribution; no pattern could be detected within the clusters of papillae. Examination of stereo pairs of these clusters further confirmed this irregular characteristic.

Spectrophotometric examination of the reflective characteristics of the purple area indicates the possible presence of a red pigment in the intermembranal matrix in addition to the three bile pigments, pterobilin, sarpedobilin and phorcabilin, identified by Bois-Choussy (1977). The reflective peaks at 470 nm (blue) and 635 nm (red) are separately observed in two of the polymorphic forms of this species, further strengthening the possibility of the presence of two pigments (Allyn and Miller, in prep.). Certain scales, when brushed from the surface, opalesce, particularly with a mauve/blue lustre which, curiously enough, tones in with the pigmented area of the membrane. This fact no doubt deceived Haugum and Samson (1980), who believed the unusual purple/mauve intermembranal pigment was located in the scales.

Physical separation of the wing membranes (figures 25-28) confirms the presence of the intermembranal matrix observed upon sectioning for TEM study (figures 35-36). When the dorsal and ventral pigmented membranes are separated mechanically, the matrix and pigmented bodies usually adhere to the dorsal portion (figures 26-28). The area (a) in figure 26 is clear and colourless, having been stripped of pigment, while the adjoining areas are the purple colour of *G. weiskei*.

The pigment deposition in the purplish pink basal blocks of the median band is markedly asymmetrical (figure 35). The granules (35a) are deposited in close proximity to the ventral membrane. This perhaps accounts for the adherence of the intermembranal pigment bodies to the dorsal membrane upon mechanical separation of the two. Curiously the pigment deposition in the post-discal green spot is similar to that found in *G. sarpedon* (figure 36). The pigment granules (36a) appear to be of similar size and shape.

**Graphium meeki inexpectatum J. & L. Miller**

The dorsal surface of the blue/green pigmented area is similar to that found in the other members of this genus, with piliform scales of approximately the same dimension as those of *G. s. sarpedon* and *G. a. agamemnon* (figure 21). The linear ridge lines are greater in number and more closely aligned than in the other species observed. Their diameter is ca. 4  $\mu$ m (figure 22). The pigment demarcation is sharp and coincident with the presence of the brown dentate scales.

The papillae of the pigmented area (figure 3) are similar to those of the other species of this genus so far examined (except for *G. weiskei*, see above).

The ventral membrane is covered with lamellate scales, the distal half of which are white and the proximal half clear. They are arranged in a single layer and no papillae are discernible on this surface.

**Graphium mendana mendana (Godman & Salvin)**

The dorsal surface is covered with piliform scales of generally the same form as those of *G. s. sarpedon*. The spacing of the scales is, on the average, less than in *sarpedon* (ca. .03 mm), but this is not believed to be significant (figure 11). These scales appear to be significantly smaller in diameter being on the average only 3.2  $\mu$ m. They contain the same twelve to fourteen ridge lines tightly aligned with extremely short, erect scutes (figure 12). The dorsal membrane papillae are also of the same form as other members of this genus (figure 4). The ventral membrane is covered with a single layer of lamellate scales which are variably transparent to white.

**Graphium mendana malaitae J. & L. Miller**

The dorsal surface is covered with regular rows of piliform scales which differ from like scales of this genus in length and form being approximately .12 mm long and spaced approximately .06 mm apart (figure 13). They have only four to five ridge lines and an average diameter of 5  $\mu$ m (figure 14). The papillae are profuse on the dorsal membrane in the pigmented areas (figure 5). The ventral membrane is unusual in that it is made up of rows of alternately piliform and lamellate scales (figure 15). Beneath these scales the pigmented area is covered with papillae in the same manner as the dorsal membrane. These dentate scales are extremely thin and transparent, thus offering little masking of the membrane pigment other than by the opalescence of their ridge lines which is, for the most part, in the same area of the visible spectrum as the underlying pigment. Figure 16 illustrates the structural details of this scale form.

**Graphium codrus auratus (Rothschild)**

The dorsal membrane is covered with regular rows of broadened, piliform scales, having a length of approximately .05 mm and a breadth of approximately .005 mm. Spacing is somewhat irregular, but is about .05 mm (figures 19 and 20). The scale sockets are distinct in this species as they have no vertical striations or buttresses and the dorsal surface is replete with papillae (figure 6). The ventral surface is covered with both piliform and lamellate scales. The latter are clear except for the distal one-third, which is white. The surface is similar to that in figure 15, except that the papillae do not appear over the entire surface. They seem to be limited to the proximal area of the pigmented portion of the membrane.

**Graphium codrus medon (C. and R. Felder)**

The dorsal membrane is covered with piliform scales which are quite similar to those found on the subspecies *auratus*, except they are broader. Their average width approximates .01 mm, or about double that of *auratus* (figures 17 and 18). The scale sockets

of *medon* are like those of *auratus* (figure 7). Papillae are present on the dorsal surface and are of the usual size and shape. The ventral membrane is covered with lamellate scales which in all respects resemble those of *auratus*. No papillae were observed on this surface.

## DISCUSSION

Most colourless membranes in butterfly wings, whether scaled or not, are smooth and lack crenulations or conspicuous irregularities, even under relatively high magnification (10,000 x). At times a number of fine, sinuous wrinkles occur, generally non-parallel, and whose inter-connections may give them a net-like appearance. In certain species of *Danaus* and *Charaxes*, these slight roughenings appear to be associated with the green colouration of the membrane. Much of the same type of surface sculpturing can be seen in SEM photos of insect integument of various species, including immature stages. In the latter cases, underlying developing cuticle and other pigment-containing material may obscure any colours produced by this slight surface wrinkling. An otherwise transparent membrane, however, might easily be tinted by such irregularities.

Opalescence can be produced in the scale membrane itself, seen in the case of *Salamis parhassus*, *Morpho catenarius* and, to a lesser degree, in *Graphium weiskei*. In the last mentioned species the opalescence only becomes visible in brushed off scales and it is then misleading similar in tone to the pinkish mauve tint of the purple (red) membrane pigment not yet identified in the wings of this species. (Choussy 1977, Allyn & Miller in prep.).

The function of the cuticular lens-like papillae in *Graphium* - which are, with few exceptions, located in those areas of the exposed dorsal wing membrane immediately above the blue pigment in the region where the scales are modified - is not known. They are usually of uniform density wherever they occur. Three exceptions to the generalization were noted. In the yellow morph of *G. mendana*, the ventral membrane is covered with the papillae (similar to those usually confined to the dorsal surface (figure 15) and contains both piliform and dentate scales. The ventral surfaces in *G. agamemnon* and *G. codrus auratus* are also partially covered with papillae. It would seem that in those species with greenish yellow or yellow pigmentation, ventral surface papillae can be present. In view of Vuillaume *et al.*'s recent investigations (see below) it is possible they serve as lenses for intensifying the blue colour reflected off the underlying pigment granules, or for focusing light onto them.

The different morphological and physiological type scales are very numerous and varied (Downey & Allyn, 1975) but it is usually considered that the flattened, leaf-like, lamellate scale is the most primitive and generalized type of lepidopteran wing covering. However, some authors (for example, Hirata & Ohsako, 1960) believe that setae, or hair-like scales, preceded them in time even in the Lepidoptera, and that flattened scales have evolved more recently.

Rothschild and Jordan (1895) referred to the dorsal blue wing pattern in *Graphium* as "scaleless" and remarked that in melanic aberrations when these areas were powdered with, or completely obscured by, an extension of black, pigmented, lamellate scales, the membranes remained green.

Hopkins (1895), however, realized that where blue/green, interlamellate pigment was visible from outside, for example in certain species of Pieridae, modified, transparent scaling was present.

Baylis (1924) first associated highly modified scales with the blue/green exposed wing membrane in papilionids and Allyn *et al.* (1981) drew attention to the great variety of such scales and the fact that this combination of blue/green pigmented wing membrane and reduced or modified scales and scale sockets occurs in scattered species in widely separated families of butterflies, such as heliconiids, papilionids and danaiids. In *Graphium*, hair-like scales replace lamellate scales in the blue/green patterned area on the dorsal surface, while usually flattened, very transparent, colourless, amber or faintly green scales (Baylis, 1924), (or a combination of both types) occur on the blue/green ventral surface of the wings, in erect to supine positions. In some cases the hair-like scales are missing,

only the sockets remaining, and it seems possible in view of the modified structure of the latter (see above) that the depletion is due to dehiscence after eclosion.

One can only speculate on how such scale modifications - all of which are directed towards exposure of the pigmented membrane - originally arose. Are the hair-like scales situated above or below these structures reduced or modified lamellate scales, or are they a primitive form of seta scale retained in these particular situations? The socket modifications in both the dorsal and ventral scales situated in the pigmented membranes and the presence of club-shaped and reduced brown scales in transition areas in *Graphium* lends some weight to the former theory. So do those scales with exised margins only.

In 1940 Weiland and Tartter identified pterobilins in various Pierid butterflies, which Rudiger *et al.* (1969) later characterised correctly as biliverdin IX $\gamma$  - a bile pigment found until now only in Lepidoptera. A relatively large number of such examples were subsequently detected in the Papilionidae (Vuillaume *et al.*, 1970), especially in the genus *Graphium* (Choussy & Barbier, 1973). From *Papilio phorcas* Bois-Choussy (1977) isolated nine blue/green pigments (11.5  $\mu\text{g}$  p. wings). She noted (pers. comm.) that pterobilins were also present in the yellow form of the butterfly. One of us (Allyn, in prep.) has found that in this species some of these green pigments are present in semi-transparent scales, not only in the membranes lying immediately below them. In *Graphium sarpedon* Bois-Choussy (1977) found the same three bile pigments, pterobilin and its two metabolites, sarpedobilin and phorcabilin. In this species sarpedobilin was the dominant form, 6.30  $\mu\text{g}$  being present in the wings. "Papilios" with fully clothed membranes, such as *P. dardanus*, *P. constantinus*, *P. zenobia* or *P. demoleus* lack these concentrations of bile pigments, and she found in such circumstances only relatively small amounts in the wings - 2  $\mu\text{g}$  or merely a trace or none at all (pers. comm.). Some of this material must have come during extraction from the green haemolymph in the wing veins - the colour of which is due to the physical mixing of yellow carotenoids and blue bile pigments. This can be well illustrated in the case of *Pieris brassicae*, which if the larva is reared on leaf-free carotenoid-free artificial diet, has blue (not green) wing veins and a blue pupa.

In *Graphium sarpedon* the concentrations of pterobilins play a conspicuous part in wing pattern and no doubt serve as inter- or intra-specific recognition signals. Seen from below, against the light sky, they are semi-transparent and provide a subtle but no doubt effective type of disruptive camouflage in flight. When at rest the underside of the wings is highly cryptic. In the vast majority of Lepidoptera, despite the presence of some bile pigments hidden in the wing membranes, visible blue colouration is a feature of the covering scales themselves.

Despite their obvious function in pattern and concealment there are certain aspects of these exposed wing membranes which excite ones curiosity and pose several interesting questions. The different and varied, but sporadic modifications of the scales which this exposure entails, seem a very elaborate evolutionary device if nothing more than a blue/green wing pattern is required. Do the bile pigments fulfill some additional function as they do in the larva of *Pieris brassicae*? Vuillaume *et al.* (1979/1981/1982) and Vallot *et al.* (1982) demonstrated that they then function as photoreceptors promoting the conversion of  $\text{O}_2$  to  $\text{H}_2\text{O}_2$ . The Breakdown of  $\text{H}_2\text{O}_2$  by catalysis is considered to be an energy releasing mechanism. Is it possible that in the exposed wing membranes pterobilins also function as photoreceptors? If so the lens-like surface structures of the grainy papillae could serve to concentrate the light in the pigmented areas, rather than increase the blue reflection as we originally supposed.

A striking feature of *Graphium sarpedon* and *G. antheus* is the paucity of carotenoids found in their tissues. Only lutein is present in both the pupal and adult stages. Thus they are the only butterflies known lacking  $\beta$ -carotene (Rothschild & Mummery, in prep.). The pupae of seven species of *Papilio* were investigated by Valadon and Mummery (1978), which, as adults, possess fully clothed wing membranes and all contained B-carotene and 3 - 10 different carotenoids with a mean of 34  $\mu\text{g}$  per individual. The pupa of *G. sarpedon* contained 8.7  $\mu\text{g}$  of lutein only. It should be recalled that when reared on an artificial diet devoid of carotenoids (Rothschild *et al.*, 1975) the pupa of *Pieris brassicae* failed to match its background and then seemed unable to respond to certain wavelengths of light (during the "sensitive period") which normally direct the withdrawal of pterobilins from

the peripheral tissues. Thus even on a white or pale yellow background it remained blue. Do carotenoids act as a deciphering system of these light cues which, via the brain, ensure the appropriate release of the hormones, which in their turn control the secretion and distribution (Ressin, 1980; Riddiford & Kiely, in press) of the pigments concerned?

We can expect that the pterobilins do not fulfill precisely the same function in the wings of all Lepidoptera. Thus Bois-Choussy (1975) has shown with the aid of radioactive tracers that in *Actias selene* the larval pterobilins are directed only into the female eggs and that those found in the wings of both sexes are synthesised *de novo* during the late pupal stage. On the other hand Vuillaume *et al.* (1979, 1981) showed that there was no synthesis of pterobilins in either the pupal or adult stage of *P. brassicae* and she regarded them in the wing membranes of this imago as waste products. It should be relatively easy to determine, for instance, whether *Danaus hamata* or *D. limniace*, which also possess exposed blue/green membranes, lack the usual carotenoids present in related Danaids (Feltwell & Rothschild, 1974) with fully clothed wings. Is this a feature of all the scattered examples of butterflies with concentrations of bile pigments in the membranes? Further research is required especially into the enigmatical relationship between carotenoids and pterobilins.

## CONCLUSIONS

1. Many species of the genus *Graphium* exhibit intermembranous blue/green and yellow/green pigments, which can be seen from outside and form part of the wing pattern.
2. Exposed blue/green intermembranous pigments are always accompanied by unique papillate, lens-like structures on the dorsal surface of the membrane.
3. Exposed yellow/green pigments (but not blue/green pigments) are also accompanied on the surface of the ventral membrane by similar papillae.
4. Exposed pigmented wing membranes on the dorsal surface are uniformly accompanied by rows of piliform scales.
5. The intermembranous pigments are contained in a stable matrix. In an alkaline medium the matrix becomes fluid and easily extractable.
6. The bile pigments are oblate-spheroid bodies in shape, approximately  $0.5 \times 0.25$  microns.
7. The microstructure of the membranes varies little between species. Only in *G. weiskei* the papillae on the dorsal surface are distinctly more conical than in related species.
8. Both the lamellate and piliform scales associated with the exposed pigmented membranes are inserted directly into the pigment-bearing matrix, lacking the bulb-like socket commonly observed in other scales.
9. It is likely that *G. weiskei* has in addition to pterobilins a red intermembranous pigment.
10. The function of pterobilins in wing membranes with a reduced scale vestiture and their relationship with the carotenoids present should be further investigated. The absence of  $\beta$ -carotene in both pupa and adult of *Graphium sarpedon* is interesting. Vuillaume *et al.*'s (1982) and Vallot *et al.*'s (1982) suggestion that larval pterobilins can act as photoreceptors is of primary importance in this connection. It could explain the morphological changes in the scales in the region of the pigment concentrations, and the presence of the minute lens-like structures situated over the pigment granules.

## ACKNOWLEDGEMENTS

We would like to express our grateful thanks to Professor Michael Barbier and Dr. Michele Bois-Choussy for permission to quote from their unpublished work, and for much help and advice.

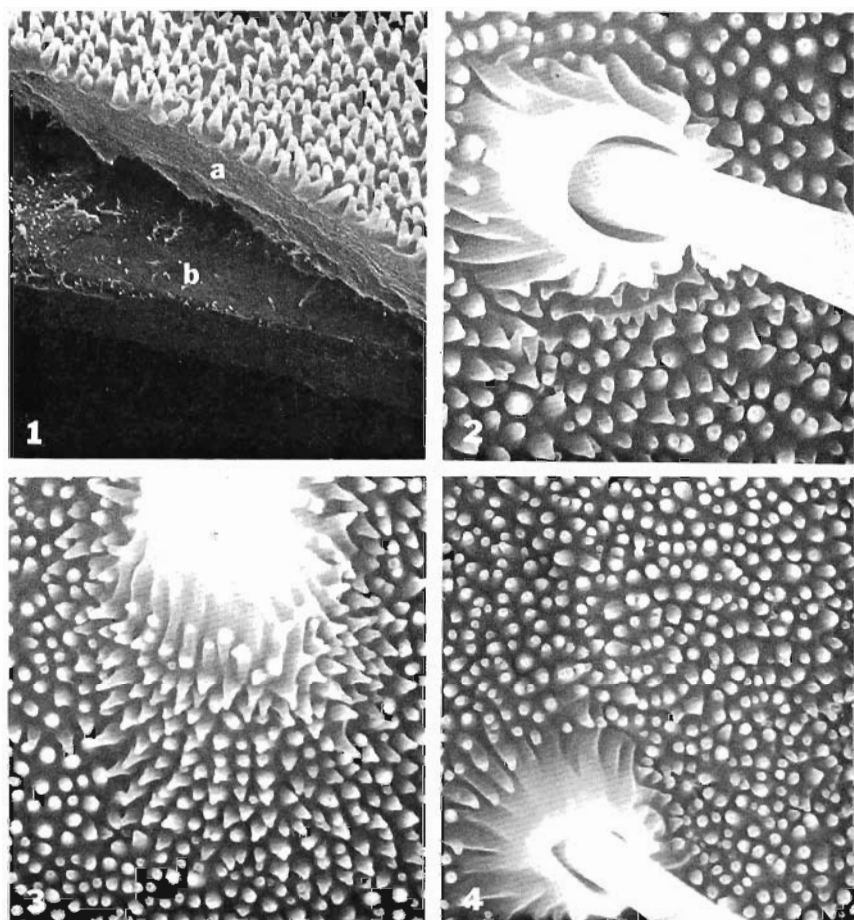
We would also like to thank Professor Monique Vuillaume and Professor Lynn Riddiford for helpful and stimulating discussion and the loan of their manuscripts before publication.

Both World Butterflies (Robert Goodden) and the Butterfly House, Syon Park (Clive Farrell) have generously supplied us with live *Graphium*.

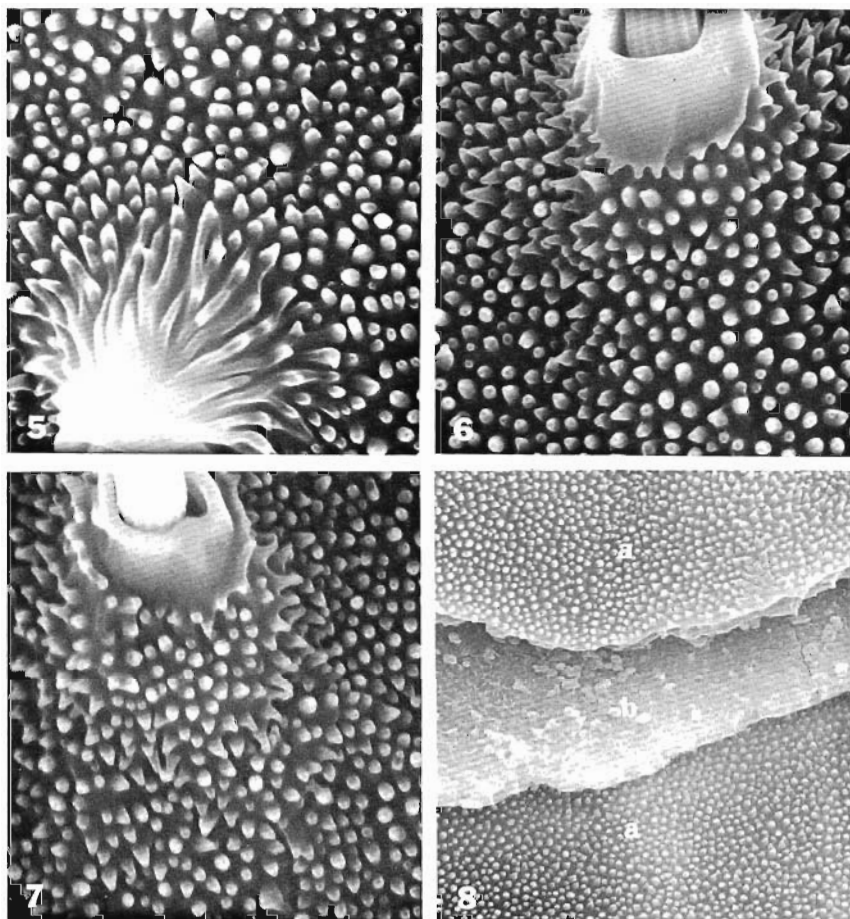
Our thanks to Dr. John C. Downey for his helpful advice in the preparation of this manuscript and to Dr. and Mrs. L.D. Miller for their critical examination of it.

## REFERENCES

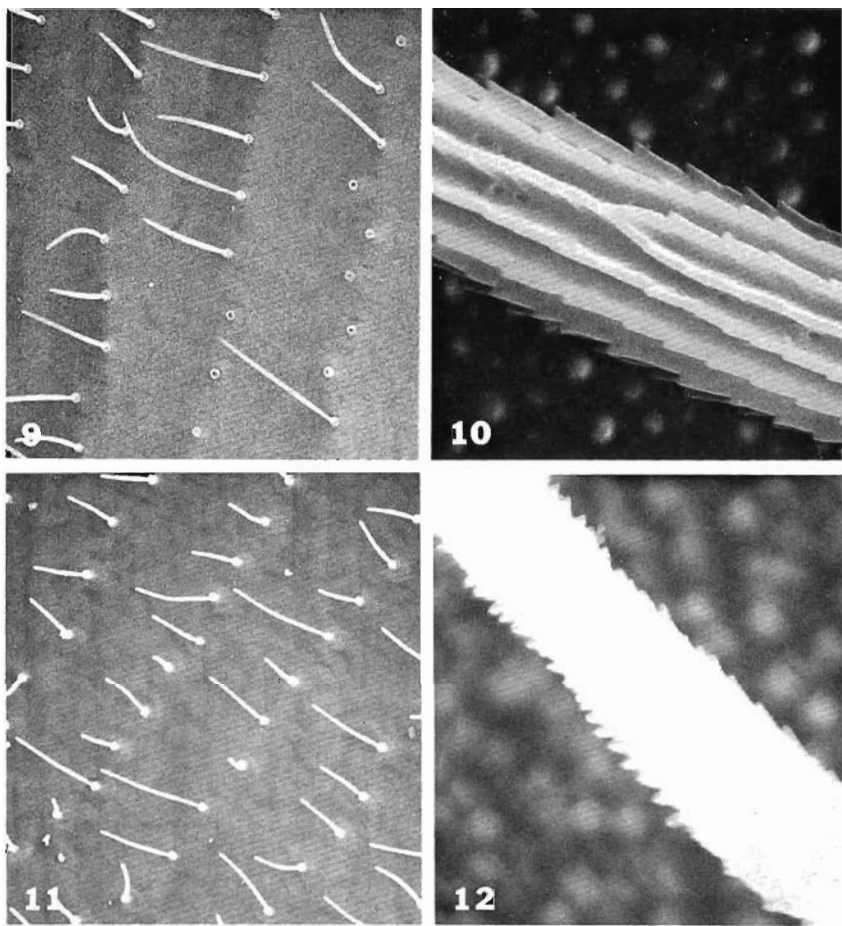
- Allyn, A.C., Barbier, M., Bois-Choussy, M. & Rothschild, M., 1981. Have the blue bile pigments in the wing membranes of the butterflies a special function? *Antenna*, Jan. 1981, Vol. 5, No. 1.
- Baylis, H.A., 1924. Colour production in Lepidoptera. *The Entomologist* 57: 2-6.
- Bois-Choussy, M., 1977. Pterobilin, phorcabilin et sarpédobiline pigments biliaires IX-v isolés de Lépidoptères. Biosyntheses, réactivités et structures. These présentée a l'Université de Paris Sud.
- Choussy, M. and Barbier, M., 1973. Pigments biliaires de Lépidoptères: identification de la Phorcabilin I et de la Sarpédobiline chez diverse espèces. *Biochemical Systematics* Vol. 1 pp 199-201.
- Downey, J.C. and Allyn, A.C., 1975. Wing-scale morphology and nomenclature. *Bull. Allyn Mus.*, 31: 1-32.
- Feltwell, J. and Rothschild, M., 1974. Carotenoids in thirty-eight species of Lepidoptera. *J. Zool., Lond.* 174: 441-465.
- Haugum, J. and Samson, C., 1980. Notes on *Graphium weiskei*. Lep. Group 68 suppl. No. 8 1980. Denmark.
- Hirata, K. and Ohsako, N., 1960. Studies on the structure of scales and hairs of insects. III. Microstructure of hairs of the butterfly, *Colias erate poliographus* Motschulsky. *Sci. Rep. Kagoshima Univ.*, 9: 123-135.
- Hopkins, F.G., 1895. The pigments of Pieridae: a contribution to the study of excretory substances which function in ornament. *Phil. Trans. R. Soc. B.*, 186: 661-681.
- Riddiford, L.M. and Kiely, M.L., (in press). The hormonal control of commitment in insect epidermis - cellular and molecular aspects. In *Regulation of Insect Development and Behaviour*, Univ. Wroclaw Press.
- Rothschild, M. and Gardiner, B., Valadon, G. and Mummery, R., 1975. Lack of response to background colour in *Pieris brassicae* pupae reared on carotenoid-free diet. *Nature*, 254, no 5501, pp. 592-594.
- Rothschild, M. and Mummery, R. (in prep.) Carotenoids of the Swallowtail butterflies *Graphium sarpedon*, *G. antheus* and *Papilio machaon gorganus*.
- Rothschild, W. and Jordan, K., 1895. A Revision of the Papilios of the Eastern Hemisphere, exclusive of Africa. *Novitates Zoologicae* 2 (3) 167-463.
- Rudiger, W., Klose, W., Vuillaume, M., Barbier, M., 1969. Sur la biliverdine IX a pigment vert des insectes orthoptères, *Clitumnus extradentatus* et *Mantis religiosa*. *Bull. Soc. Chem. Biol.*, 51: 559-562
- Ressin, W. J., 1980. The effect of juvenile hormone on pupal pigmentation of *Pieris brassicae* L. *J. Insect Physiol.* 26: 295-302.
- Valadon, L. R. G. and Mummery, R. S., 1978. A comparative study of carotenoids in *Papilio* sp. *Comp. Biochem. Physiol.* 61 B: 371-374
- Vallot, R., Bona, X., Buvet, R., Vuillaume, M. & Calvayrac, R., 1982 (in press). Electrochemical behavior of pterobilin, and insect tetrapyrrolic pigment, on graphite paste electrode. *Bioelectrochemistry and Bioenergetics*, 1.
- Vuillaume, M., Choussy, M. and Barbier, M., 1970. Pigments tétrapyrroliques verts et bleus des ailes de Lépidoptères pterobilin et néoptérobilines. *Bull. Soc. Zool. France* 95: 19.
- Vuillaume, M., Calvayrac R. and Best Belpomme, M., 1979. Photo conditioning in the integument of *Pieris brassicae*: ATP production *in vivo* and *in vitro*. Properties of the system. *Biologie Cellulaire*, 35: 71-80.
- Vuillaume, M., Best-Belpomme, M. & Calvayrac, R., 1981. Biological model for the possible importance of hydrogen peroxide. 6th International Symposium of bioelectrochemistry and bioenergetics, Kiryat Ananim, Israel.
- Vuillaume, M., Calvayrac, R., Hubert M., Braiand, J. & Best-Belpomme, M., 1982 (in press). Hydrogen peroxide and catalase activity in the integument of an insect (*Pieris brassicae*). *Biol. Cell.* 43(3).
- Wieland, H. and Tarter, A., 1940. Über die Flügelpigmente der Schmetterlinge VIII. Pterobilin, der blaue Farbstoff de Pieridenflügel. *Annalen der Chemie*, 545: 197-208.



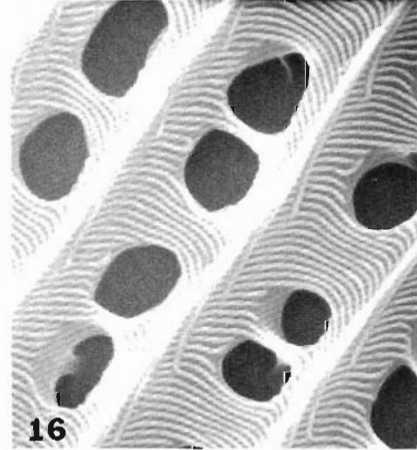
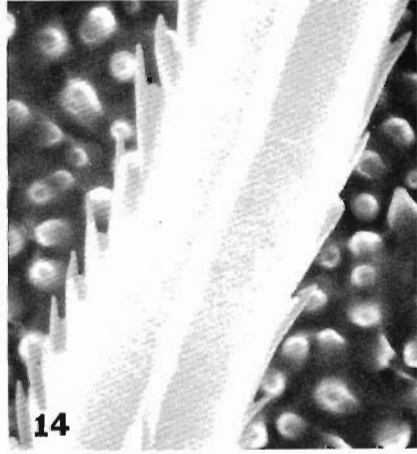
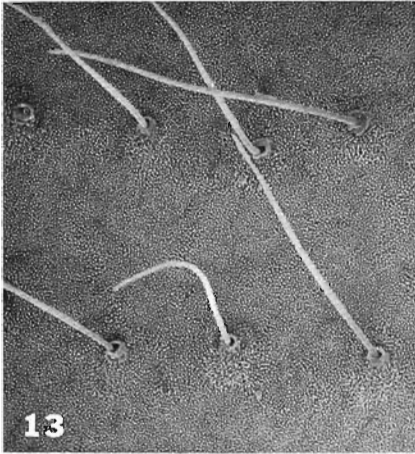
Figures 1-4: Pigmented membranes. 1: *Graphium sarpedon*; (a) dorsal membrane, (b) ventral membrane (5000x). 2: *G. agamemnon*; dorsal membrane (3700x). 3: *G. meeki inexpectatum*; dorsal membrane (2800x). 4: *G. mendana mendana*; dorsal membrane (2800x).



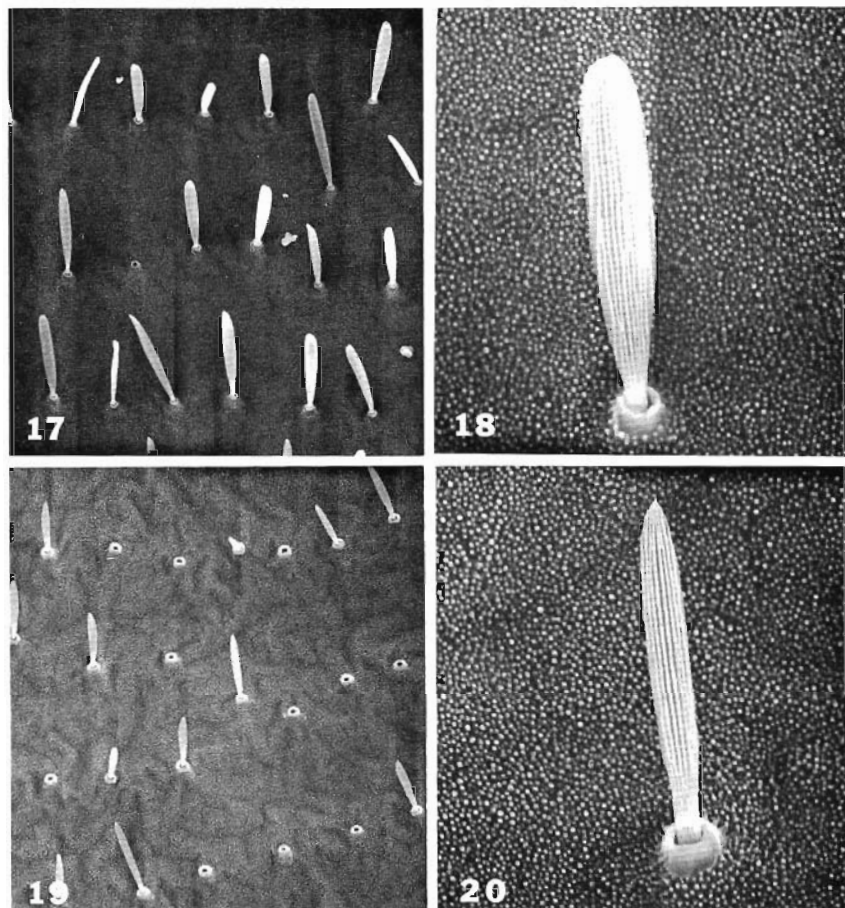
Figures 5-8: Pigmented membranes. 5: *Graphium mendana malaitae*; dorsal membrane (2800x). 6: *G. codrus auratus*; dorsal membrane (2800x). 7: *G. codrus medon*; dorsal membrane (2800x). 8: *G. meeki inexpectatum*; (a) dorsal membrane, (b) ventral membrane (1200x).



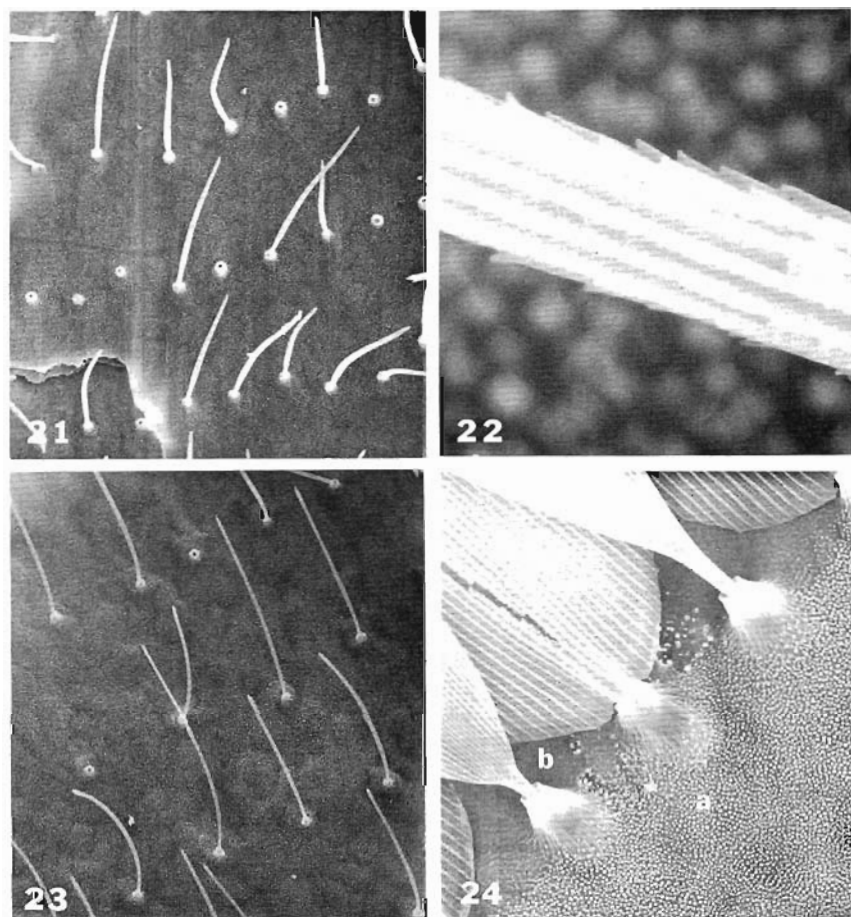
**Figures 9-12: Modified scales.** 9: *Graphium agamemnon agamemnon*; dorsal surface of pigmented membrane (190x). 10: *G. a. agamenon*; piliform scale (6300x). 11: *G. mendana mendana*; dorsal surface of pigmented membrane (190x). 12: piliform scale (6300x).



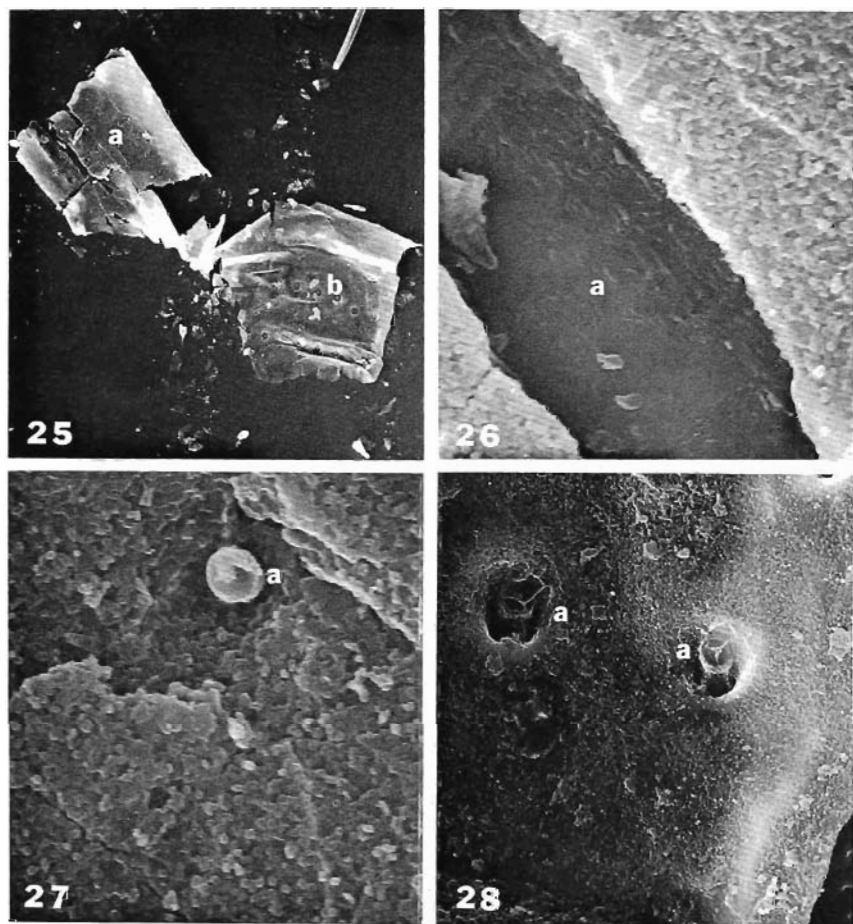
**Figures 13-16:** Modified scales. 13: *Graphium mendana malaitae*; dorsal surface of pigmented membrane, (280x). 14: *G. m. malaitae*; piliform scale (6300x). 15: *G. m. malaitae*; ventral surface of pigmented membrane (190x). 16: *G. m. malaitae*; transparent scale of ventral pigmented membrane (6300x).



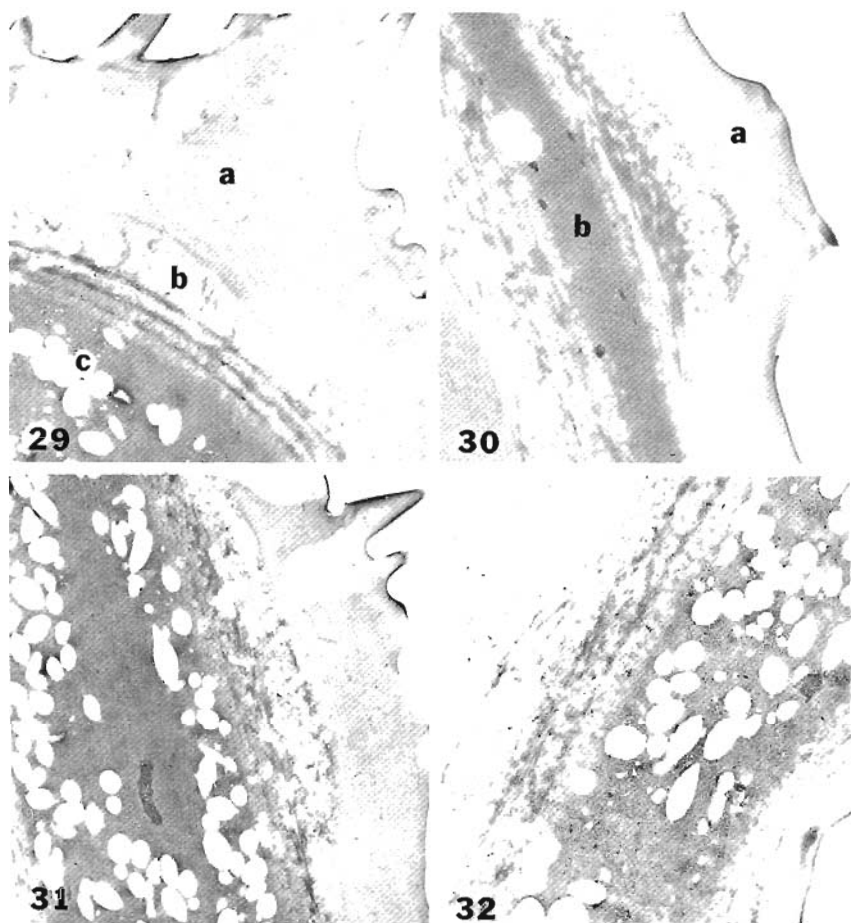
**Figures 17-20: Modified scales.** 17: *Graphium codrus medon*; dorsal surface of pigmented membrane (190x). 18: *G. c. medon*; modified scale (930x). 19: *G. codrus auratus*; dorsal surface of pigmented membrane (190x). 20: *G. c. auratus*; modified scale (930x).



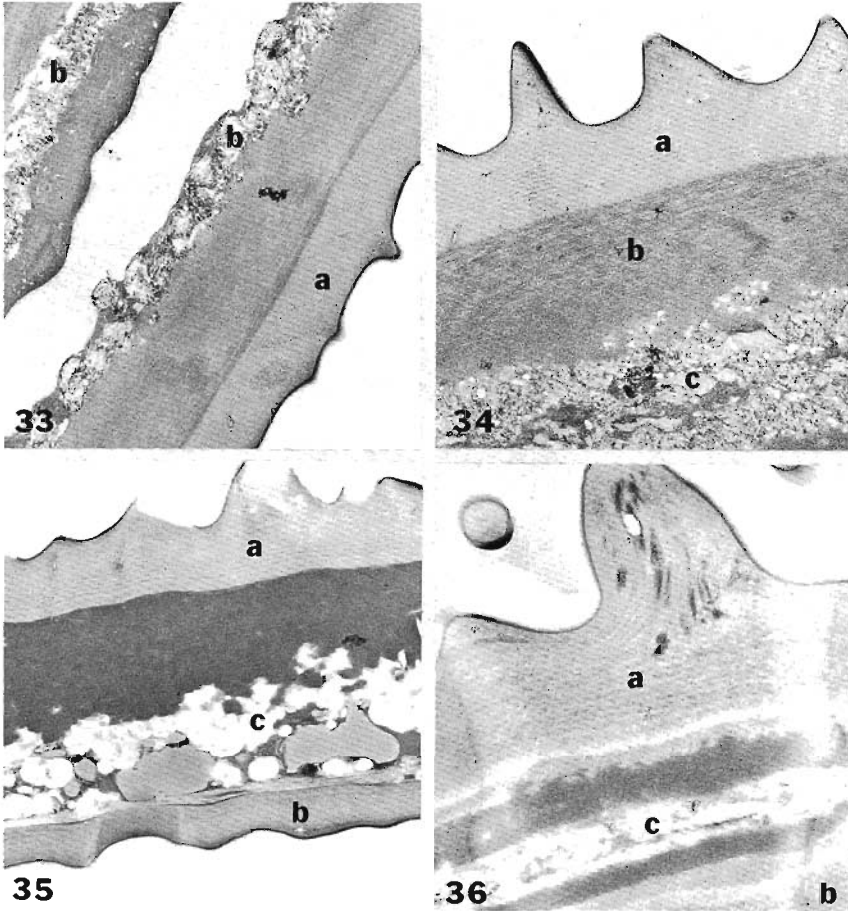
**Figures 21-24:** Modified scales. 21: *Graphium meeki inexpectatum*; dorsal surface of pigmented membrane (190x). 22: *G. m. inexpectatum*; piliform scale (6300x). 23: *G. weiskei*; dorsal surface of pigmented membrane (190x). 24: *G. mendana mendana*; demarcation line of pigmented membrane (a) and clear membrane (b) (630x).



Figures 25-28: Split wing membranes of *Graphium weiskei*. 25: separated wing membranes; (a) dorsal membrane interior aspect; (b) ventral membrane interior aspect (63x). 26: interior of dorsal membrane; (a) portion of membrane without interstitial material and having no color (1900x). 27: interstitial material containing pigment bodies adhered to dorsal membrane; (a) piliform scale socket (630x). 28: interior of ventral membrane; (a) scale sockets (630x).



Figures 29-32: Sectioned wing membranes of *Graphium sarpedon*. 29: dorsal cuticle; (a) expanded laminations of surface projections, (b) helicoidal microfibrils, (c) pigment bodies (10,000x). 30: ventral cuticle; (a) reduced laminations, (b) near absence of pigment bodies in areas between projections (17,000x). 31: cuticle section illustrating concentration of pigment bodies (19,000x). 32: cuticle section similar to figure 31 (17,000x).



Figures 33-36: Sectioned wing membranes of *Graphium agamemnon agamemnon* and *Graphium weiskei*. 33: *G. a. agamemnon*; ventral cuticle, (a) lamellate cuticle, (b) pigment bodies (12500x). 34: *G. a. agamemnon*; dorsal cuticle, (a) lamellate cuticle, (b) non-lamellate cuticle, (c) cellular remnants including pigment granules (16000x). 35: *G. weiskei*; dorsal (a) and ventral (b) cuticles of purple pigmented area, showing asymmetrical deposition of pigment granules (c) (9500 x). 36: *G. weiskei*; dorsal (a) and ventral (b) cuticles of green pigmented area showing symmetrical deposition of pigment granules (c) (22000x).

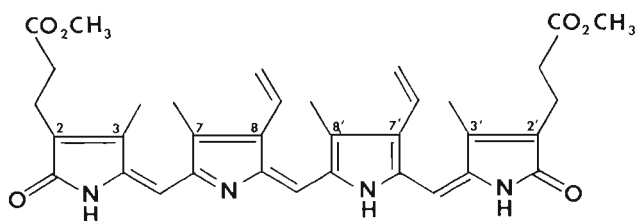


Figure 37: *PTEROBILINE* (14) (biliverdine IXy)

This public document was promulgated at a cost of \$814.50 or \$1.36 per copy. It makes available to libraries, scholars and all interested persons the results of researches in Entomology.