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OBSERVATIONS ON MALE U-V REFLECTANCE AND SCALE ULTRASTRUCTURE IN PHOEBIS (PIERIDAE)

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The genus *Phoebis* and its relatives are medium to large sized sulphurs limited to the new world. Their colors, as with many pierids, range from white to yellow, orange and red, and mixtures of these. While most of the visible color is probably due to pigments in the wing scales, some species show a brilliant iridescence in the near ultra-violet range of the spectrum, which is apparently caused by optical interference in a lamellar system found on the ridges of the outer wing scales. The latter ultrastructure has been described in other Pieridae: in *Eurema* by Ghiradella *et al* (1972); *Colias* by Hirata *et al* (1957, 1959, 1960), by Silberglied and Taylor (1973) and by Ghiradella (1974); and in *Pieris* by Obara and Hidaka (1968). Sellier (1971) published an SEM photograph of *Genopteryx* scales without further comment. In a series of papers Descimon (1965, 1966a, 1966b, 1969, 1971, 1976) contributed significantly to the understanding of pigment formation in the Pieridae, and to the cytoplasmic ultrastructure of scales. About 25 pierid species have males in which U-V reflectance has been indicated, mostly by photographic techniques. Mazokhin-Porshnyakov (1954, 1957, 1969) studied the U-V reflecting properties of representatives of several families of butterflies, but did not relate color to scale type. He recorded reflectance percentages and patterns in five wing areas of several species including two *Phoebis* (*philea* and *argante*) and concluded that the U-V reflectance was "undoubtedly perceived" by insect eyes.

Since *Phoebis* has marked color variants and overlapping forms in both sexes in most of its species in both the U-V and the visible parts of the spectrum, it was our feeling that it would be an excellent study group in which to attempt to relate reflectance and scale ultrastructure. Further, since the male wings show marked color reflectance, but seem to be less variable in color characters than the females of *Phoebis* species, we hypothesized that the contribution of scale ultrastructure to color might be more easily sought in the males.

Brown (1929, 1931: 10) was of the opinion that *Phoebis* and its close relative *Aphrissa*, are very recently evolved genera, which show much diversification under varying environmental conditions. Knowledge of the nature and origin

of color production in *Phoebis* will not only contribute to the taxonomic and evolutionary concepts within the genus, but will broaden our understanding of the biological significance of ultrastructure and its contribution both to general morphology and to behavior.

Over 50 years ago Mattram and Cockayne (1920) noted that scales contained "fluorescent pigments" in the ultraviolet part of the spectrum which are not visible to the unaided human eye. They suggested (*loc. cit.*: 38-39) that the fluorescent dimorphism between the sexes aids in sexual recognition. Cockayne (1924) demonstrated U-V reflectance in a wide variety of lepidoptera.

Except for studies such as Koehler (1941) where U-V light was used as an irradiating source to study induced variation, it is somewhat surprising that after Cockayne's introductory work, there was a thirty-year gap in studies involving U-V reflectance and possible advantages to the species. In the late 1960's and early 1970's, there has been an increasing number of studies on ultraviolet reflectant scales, and their optical properties.

Since 1965 when Nekrutenko first suggested and used ultraviolet reflectance photography as a taxonomic tool, it has been used in several pierid groups (Ferris, 1972, 1973; Silberglied and Taylor, 1973; Scott, 1973, Nekrutenko, 1970a, 1970b, 1973). Ultraviolet video-viewing has also been suggested as an exciting teaching aid (Eisner *et al*, 1969: 1174). Photographic techniques have also been used in detecting gynandromorphs (Nekrutenko, 1966.)

MATERIALS AND METHODS

Most of the described taxa in the genus were closely examined visually and photographs were taken of select species using appropriate filters (see below) to cut out all but the near ultra-violet wavelengths.

Based on both visual color and U-V reflectance properties, we eventually selected three species for detailed SEM studies: *Phoebis philea* (Linn.), *P. thalestris* (Illig.) and *P. avellaneda* (Herr-Schaef.). These had an increasing component of orange and red scales from the almost monotone lemon-yellow phase of *philea*, through the heavily infused orange-yellow of *thalestris* to the red cast of *avellaneda*, where the yellow scaling is reduced to a relatively small fraction of the visual component. Further, the U-V reflectance patterns on the upperside of these three species have consistent and significant differences. We were convinced that differences of the magnitude observed between them would also be observable with scanning electron microscopy if they were correlated in any way with ultrastructure.

Hirata and Kubota (1957) noted that some differences between their observations on ultrastructure in *Colias* and those of other authors were explained by the methods used, rather than just the specimens involved. It thus seemed advisable to briefly note the procedures and equipment used so that results could be consistently duplicated for interpretation.

Reflectance and absorbance were measured on a Beckman (DB-GT) double-beam grating spectrophotometer with a 10° specular reflectance accessory. A Beckman 10-inch recorder charted samples in a continuous readout from 700 nm to 190 nm. The SEM instrument used was a JSM-U3. Samples were coated with 60/40 gold-palladium in a DE10 vacuum evaporator. Dried museum specimens were used.

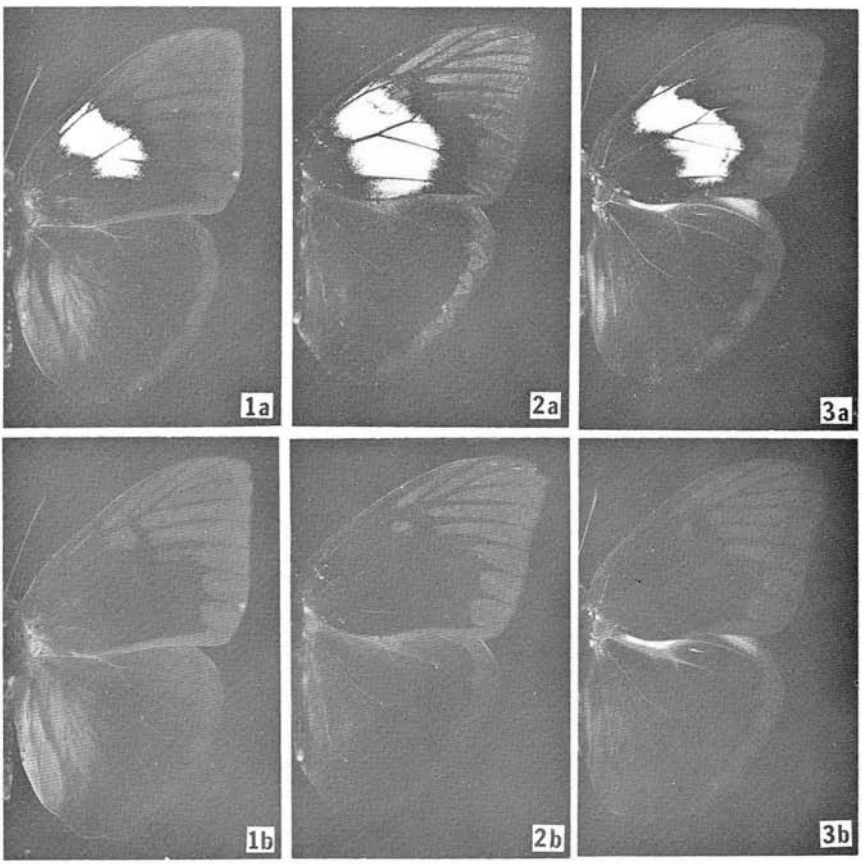
Separation photographs were made with Wratten tight-band filters numbered 18a, 50, 75, 73 and 72b. Exposure times were balanced to a standard gray scale using Panatonic X film, which is essentially insensitive to deep red and infra-red (650 nm and higher). Originally such photographs permitted determination of the dominant frequencies in specific wing areas. One millimeter square samples were then excised from areas (see Fig. **4**), color photographed and enlarged to 200 diameters for precise orientation of scales in SEM examination. As techniques were perfected, much of this original orientation procedure was shortened, particularly as expertise was gained at recognizing specific areas, colors, and scales.

The ultra-violet photographs were taken with an 18a Wratten filter using a 25w/s ring flash. Focal length was increased 3 mm to compensate for focal length difference in the shorter wavelengths. Scale vocabularly follows that given in Downey and Allyn (1975).

RESULTS AND DISCUSSIONS

Photographic and Spectrophotometric Comparisons

Photographs "**a**" through "**e**" were taken with Wratten tight-band filters of *Phoebis philea* (Fig. **1**), *P. thalestris* (Fig. **2**) and *P. avellaneda* (Fig. **3**). Figures **1a, 2a** and **3a** were obtained with an 18a filter which peaks at 360 nm (310-400 nm). A comparison of the three figures shows the similarities and differences in the patterns of reflectance in the near ultra-violet range for the three species. Note that *thalestris* has the largest of the high intensity reflective spots in the forewing. The scent patch in the costal area of the hindwing of *avellaneda* (Fig.

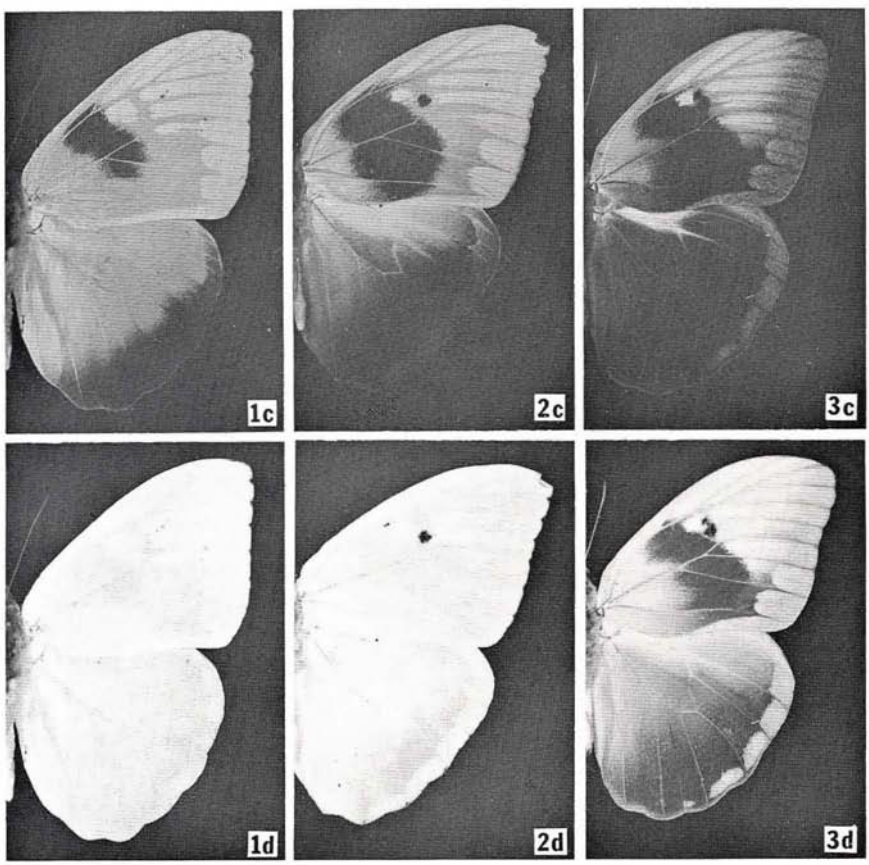


Dorsal surface of male *Phoebis* photographed using tight-band filters. Figs. 1, *P. philea;* Figs. 2, *P. thalestris;* Figs. 3, *P. avellaneda.* Photos (a) were taken using an 18a Wratten filter with a peak at 360nm. (Range: 310-400nm). Photos (b) were taken using a 50 Wratten filter with a peak at 450nm. (Range: 430-480nm).

3a) shows U-V reflectance with a peak at 270 nm, compared to the 350 nm peak (see below) of the forewing patch. The scent patches of the hindwings are not visible in Figures **1a** and **2a** because they are covered by the forewing. Scent patches of all male *Phoebis* on the ventral forewing, as well as the dorsal hindwing, show U-V reflectance at approximately the same intensity, but were not included in the present report.

The actual U-V reflectance for these wings may be noted in Fig. **1f, 2f,** and **3f.** Graphs were made from samples from the forewing discal cell, and oriented in the same direction in a grating spectrophotometer so that the readings might be more comparable. All three species were similar in having the peak intensity of U-V at 350 nm. Minor peaks below this wavelength are shown in all species with *thalestris* showing an unusually high intensity peak at 310 nm.

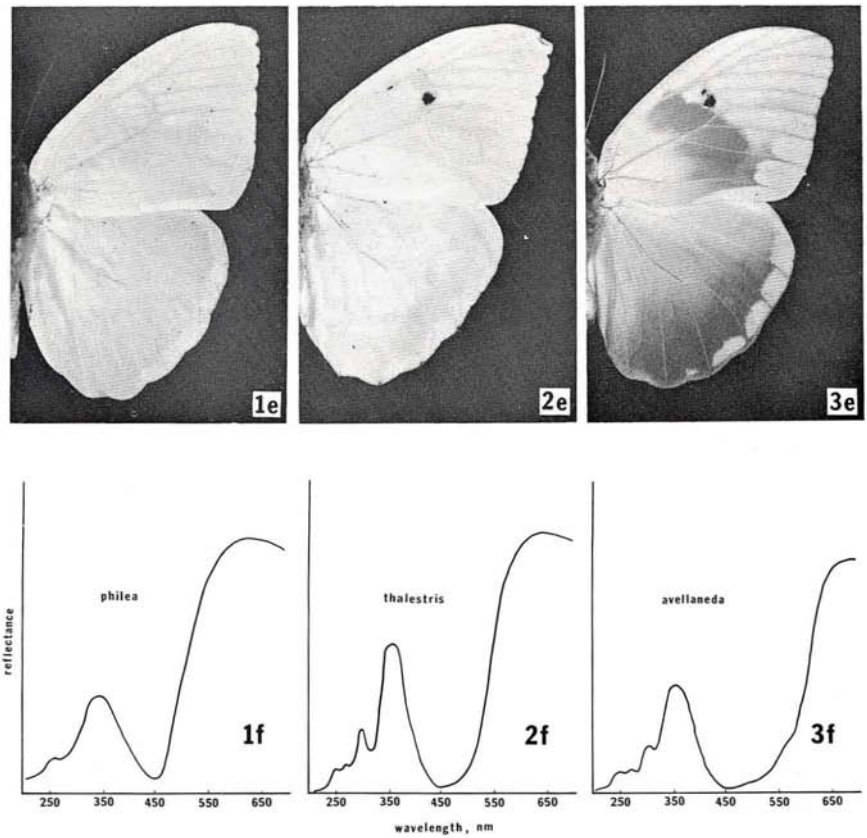
The graphs in Figures **1f, 2f,** and **3f** also clearly reflect the light frequency output obtained from the discal spots of the three species in the visible parts of the spectrum. The heavy yellow component in *philea* (Fig. **1f**) is apparent, as is the shift toward the red end of the spectrum in *avellaneda* (Fig. **3f**).



Dorsal surface of male *Phoebis* photographed using tight-band filters. Fig. 1, *P. philea;* Figs. 2, *P. thalestris;* Figs. 3, *P. avellaneda.* Photos (c) were taken using a 75 Wratten filter with a peak at 490nm. (Range: 450-540nm). Photos (d) were taken using a 73 Wratten filter with a peak at 576nm (Range: 550-600nm).

A comparison of the photographs taken with the various tight-band filters establishes an unmistakable pierid pattern, particularly in marginal and submarginal areas. One can note in the "**a**" photos for example, that each of the three species has a minimum of four similar reflectance areas in the near ultra-violet part of the spectrum: 1) the high intensity discal area, referred to above; 2) less intense, but reflective scent patches (some not visible); 3) a marginal to submarginal "glossy" area, which shows a very low intensity reflectance comparable perhaps to that shown on many wings veins, and 4) dark areas which are U-V absorbing. Clearly one could select scale samples from the same regions from each of these species, and expect that they might be similar in those ultrastructural characters which may account for this reflectance. By studying each of the "tight-band" photos, careful selection of sample areas would maximize the chances of relating color to scale structure.

Figure **4** shows the wing areas from which scale samples were taken for ultrastructural comparison on the scan electron microscope (SEM). It may be noted that the majority of reflectance patterns observed in the photographs are included



Dorsal surface of male *Phoebis* photographed (e) using a 72b Wratten filter with a peak at 605nm. (Range: 590-650nm). Fig. 1, *P. philea;* Fig. 2, *P. thalestris;* Fig. 3, *P. avellaneda.* Reflectance curves (f) of the forewing discal cells. Figures (f) are spectrophotometric records for the three species.

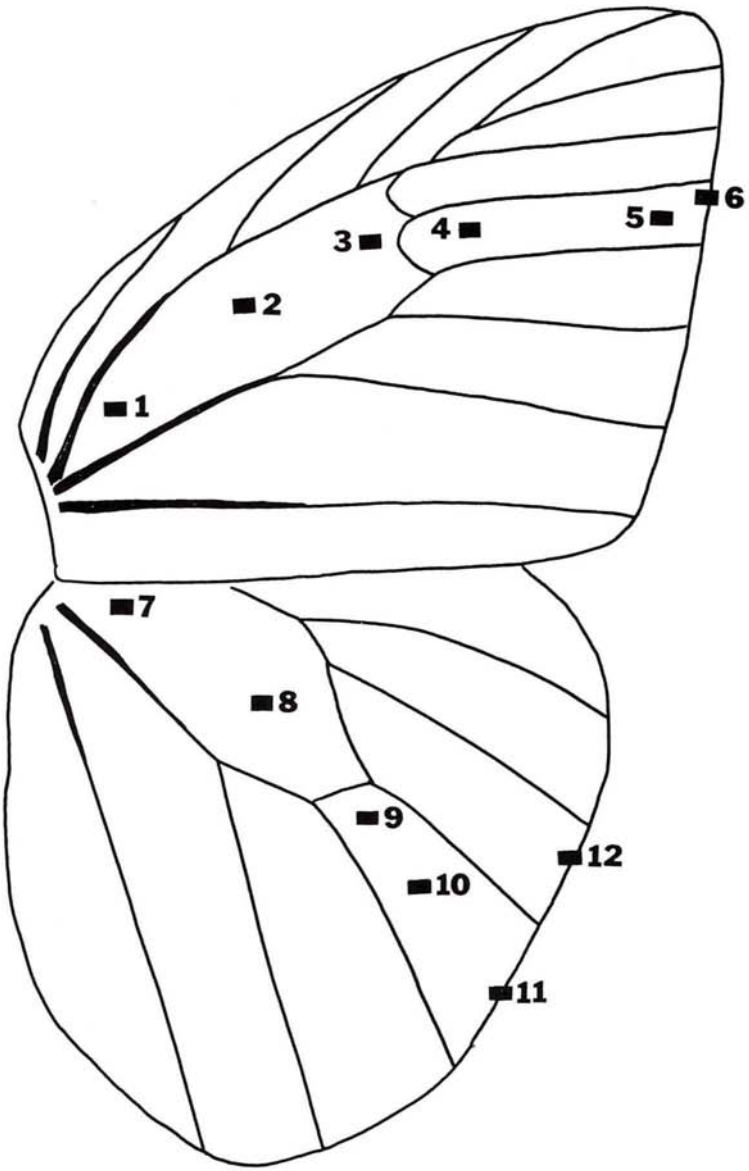


Figure 4, Wing areas from which scale samples were taken for comparative SEM analysis.

in the samples.

The U-V reflectance photographs of males of a number of *Phoebis* species and near relatives including the allied genera (considered subgenera by some authors) *Aphrissa, Rhabdodryas* and *Prestonia* are given in Figs. **5a, 5b** and **5c**. For a more accurate wavelength comparison, spectrophotometric plots of the refectivity from the same region in the discal cell of each species is presented by each graph. The absence of U-V reflectance in *Phoebis sennae marcellina* and *Aphrissa boisduvali* males may be noted on both photographs and charts. This fact, together with the occurrence of high intensity U-V reflection from females of some species, *i. e. philea,* means that this type of sexual dimorphism is not uniform throughout the genus.

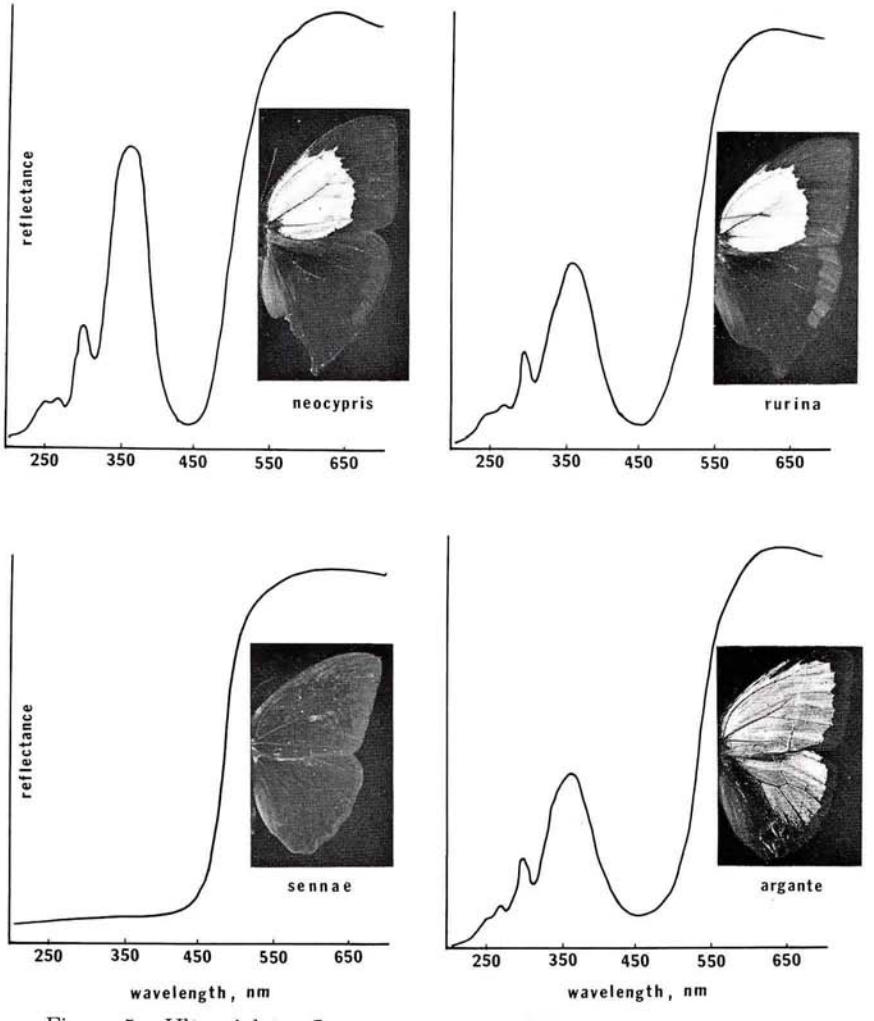


Figure 5a: Ultraviolet reflectance patterns of the upperside of representative males of the genus *Phoebis* and accompanying spectrophotometric records of samples from comparable forewing areas.

The spectrophotometric measurements were made using a constant incidence of reflection (10°) so that structural color shifts as predicted and reported by Huxley (1975) would be avoided. The 10° incidence of reflection should provide a minimum color shift.

Structural color produced by scale ridges, for example, should exhibit a variable intensity according to the angle of placement of the ridge with respect to the plane of the light path. An examination of the spectrum of the blue iridescent scales of *Morpho menelaus* exhibited this phenomenon in both the visible and ultra-violet portions of the spectrum. The maximum reflectance occurred when the ridge-line of the scales was in the same plane as the light path, and lower reflectance was obtained when the ridge-line was at right angles to the light path.

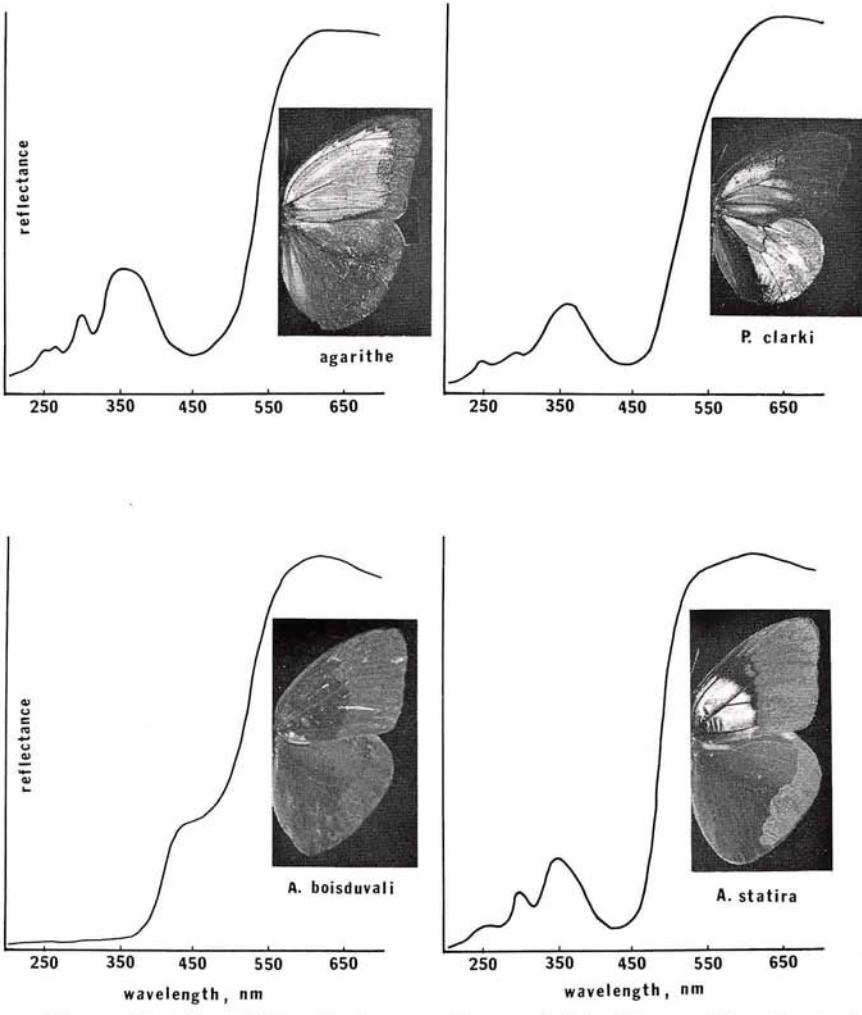


Figure 5b: Ultraviolet reflectance patterns of *Phoebis agarithe*, *Prestonia clarki*, and the related genus *Aphrissa*. The accompanying graphs are the spectrophotometric records of samples from comparable forewing discal areas.

As noted above, we were cognizant of structural colors produced in the longitudinal ridges of Pieridae, and we expected this in *Phoebis*. However, there was no observable difference in intensity in the visible portion of the spectrum when the wing was placed in various positions; only in the U-V part of the spectrum did we obtain significantly different readings depending on the angle of the wing to the plane of the light source. It seemed reasonable to conclude, therefore, that the visible colors (yellow, orange, and red) stemmed from locations in the scale below the ridges, or perhaps in the ridge sinuses, and that the ridges of these scales produced the U-V reflectance. The ultrastructure of ridges will be discussed below. However, of some interest is the fact that there was no significant difference in readings obtained between 90° and 270°, and between 0° and 180° to the plane of the light source. This would indicate that the slant of the ridge shelves has no apparent effect on the intensity of output, at the 10° incidence of reflection in this genus.

SEM Comparisons

Details of the ultrastructure of scales were studied from 12 comparable wing areas of the dorsal surface of male *Phoebis philea*, *P. thalestris* and *P. avellaneda*. In addition, samples were taken from the undersurface, from the wings of females and from specimens obtained from many different geographical areas, to insure a broader estimate of parameters of variability.

A minimum of two categories of scales occur in all wing samples observed: these were grouped by the horizontal layering of the supine scales into *covering* scales and *basal* scales. In proximal regions of the wing (extending almost to submarginal areas) the sockets of covering and basal scales are arranged in transverse rows; in the marginal area adjacent socket rows lie side by side forming an irregular, semi-tiered group of sockets. Each row is composed of alternate sockets of cover and basal scales; however, the blade of the cover scale is so wide that

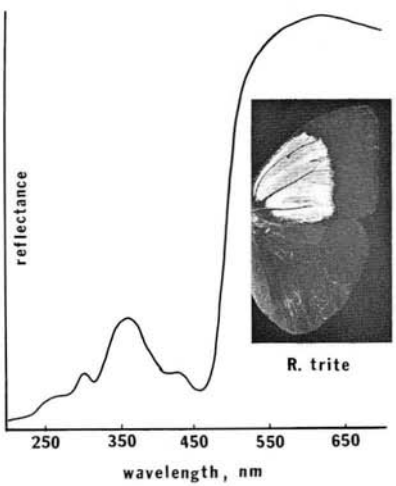
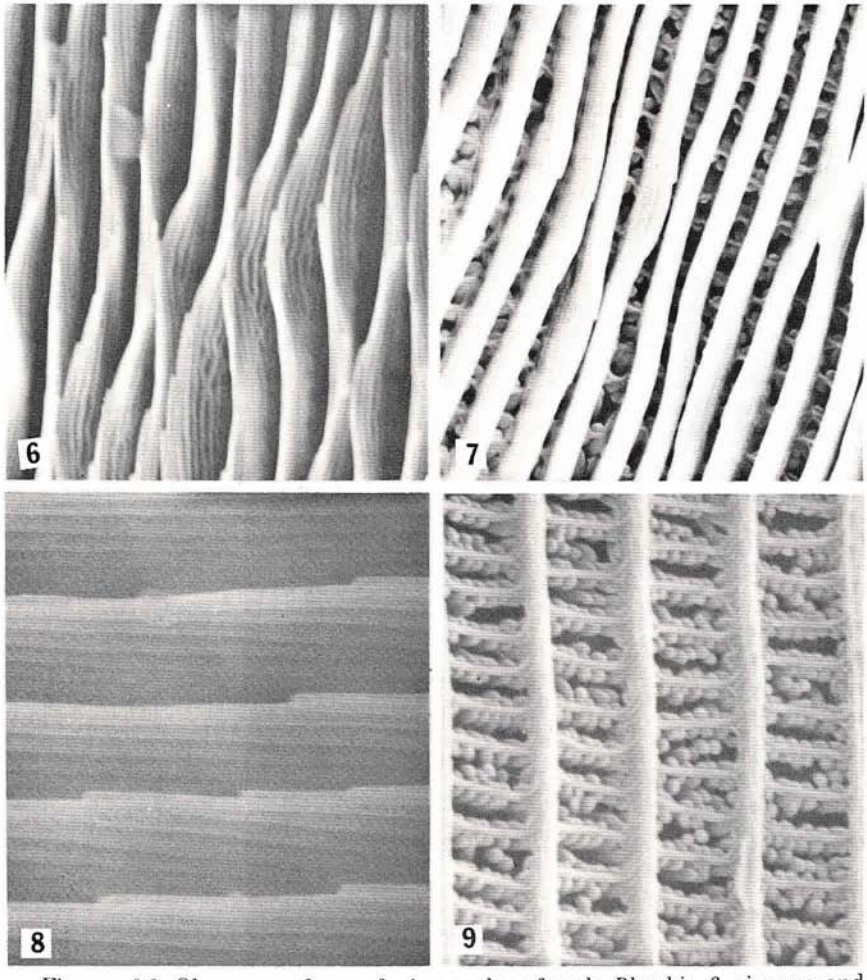


Figure 5c: Ultraviolet reflectance pattern of *Rhabdodryas trite* and accompanying spectrophotometric record of a sample from the forewing discal area.

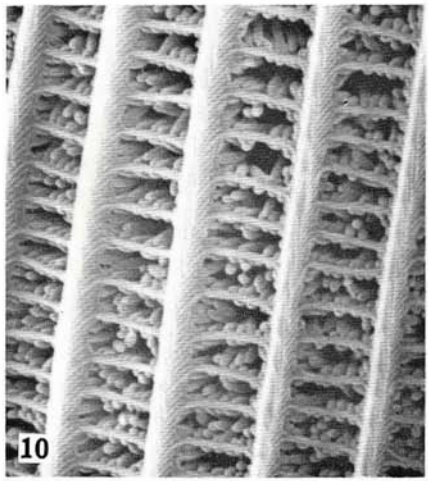
their lateral margins touch the margins of other cover scales in the same line, and overlying the intervening basal scale. The latter are often shorter than the former, so that in most basal and discal areas of *Phoebis* wings the surfaces of only cover scales are visible when viewed dorsally. Sockets of "specilized" scales such as basal "hair" scales and marginal (border) black scales may be included along and between regular tiers.

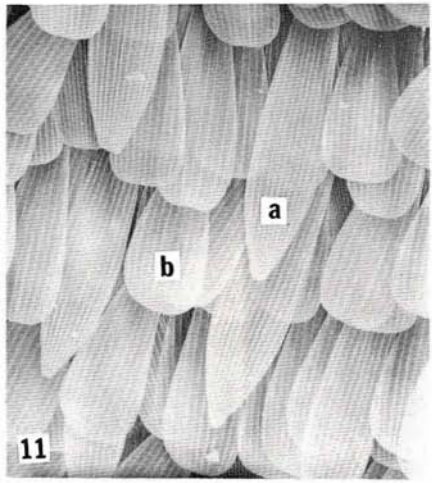
Sugmarginal and marginal areas in *Phoebis* have less distinct layering, and scales of intermediate position (the *Mittelschuppen* of Kuehn and Henke, 1929) are the norm; often they are indistinguishable in type from cover scales above or basal scales below, but admixtures of several scale types are not uncommon in this region.

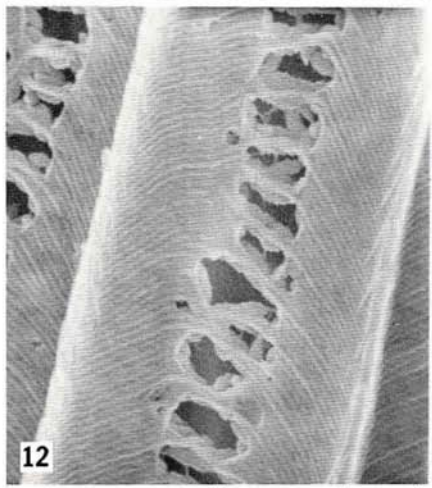


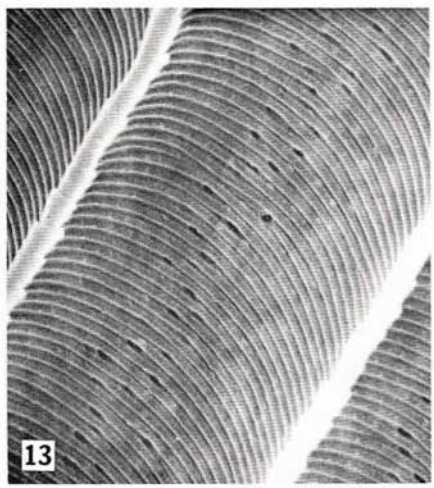
Figures 6-9: Obverse surfaces of wing scales of male *Phoebis*. 6, sinuous and elevated ridges of U-V reflective scales from discal cell showing shelves on lateral ridge surfaces (6500x). 7, U-V reflective scale showing ovoid bodies beneath cross-ribs. (6500x). 8, lateral view of U-V reflective ridges (19600x). 9, basal scale from U-V reflective discal cell (6500x).

Pigment scales (Figs. **9, 10**). Yellow-colored scales, and variations (light yellow, lemon-yellow and yellow-range) occupy the greater part of the wing surface and give each species its characteristic color. These scales show considerable variation in their length and width, and more markedly, in characters of their apices, whereas the apices of adjacent yellow scales may not alter abruptly, every individual examined has transitions from an obtuse, gently rounded apex, to the serrate condition with a varying number of incisions and denticles. Other studies (see Downey and Allyn, 1975) indicate that environmental factors may effect scale shape and the nature of the apex. However, in spite of this superficial difference, these scales do not show fundamental differences in their ultrastructure as observed with the SEM. We have not as yet been able to distinguish significant differences in the pigment bodies of these scales (see below).



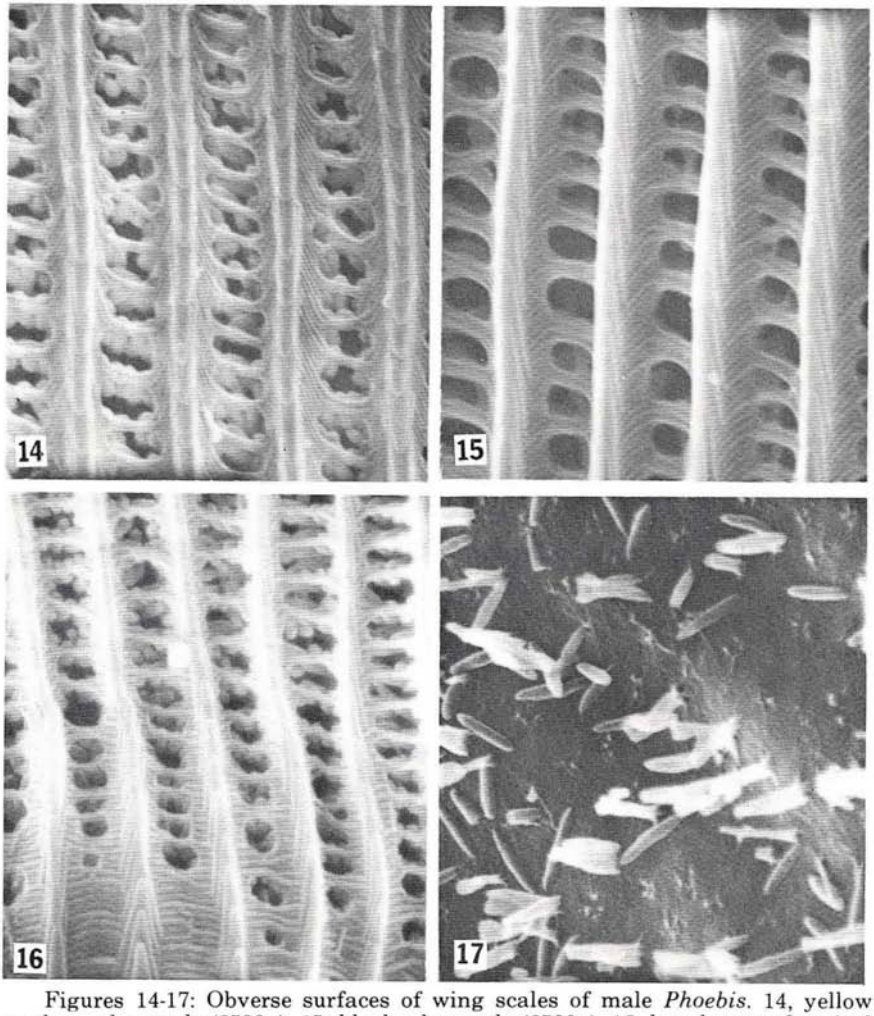






Figures 10-13: Obverse surfaces of wing scales of male *Phoebis*. 10, cover and basal scales of U-V non-reflective areas. (6500x). 11, scale forms in lightly U-V reflective areas. (200x). 12, ultrastructure of elongate scale (Fig. 11a) (6500x). 13, ultrastructure of spatulate scale (Fig. 11b) (6500x).

Pigment scales, with cross ribs showing a ladder-like arrangement (the *Leitertypus* scale of Suffert, 1924) are shown in Figure **9.** They formed the cover and the basal scales (even though different in outline) from sample areas 1, 4, 7, 8, 9, 10 and 11, in *philea, thalestris* and *avellaneda.* Some sample areas in the three species had different scale types; we had anticipated greater differences than observed. Area 3 differed in all 3 species, as suggested in Figures **1a, 2a,** and **3a,** and in *philea* the cover scales in this area were of the ladder-like, pigmented type. Area 6 was the only other sample site with major differences in scale types for the three species above; the *thalestris* sample 6 underscale was of the ladder-like type.



Figures 14-17: Obverse surfaces of wing scales of male *Phoebis.* 14, yellow or clear edge scale (6500x). 15, black edge scale (6500x). 16, basal area of typical non U-V reflective scale showing various window forms (6500x). 17, typical scale with the ridge surface removed showing trabecular fragments and displaced ovoid bodies on the inner surface of the ventral side of the scale (6500x).

Pigment scales were the principal scale types and formed the cover scales of the male underside. The cover scales in females of the three species, even in the area of the discal cell corresponding to the male U-V reflectant spot, were of this ladder-like type.

The most prominent morphological feature of U-V reflection scales (Figs. **6, 7, 8**) is the great height of the elevated longitudinal ridges; in *Phoebis philea* they average 1.8 microns elevation above the surface of the obverse membrane, compared to a height of 0.7 microns for the longitudinal ridges of the non-reflectant, ladder-like scales. The ridges of the reflectant scales are also closer together (a greater number per unit width) averaging 0.85 microns apart as measured crest to crest, compared with 2.13 microns between the crests of adjacent ridges in non-reflectant scales. Put in other terms, there are more than twice as many ridges per unit scale width in highly U-V reflectant *Phoebis* cover scales as occur in non-reflectant, or basal scales. The ridges in the former are twice as high as the width of their inter-ridge channels.

Ridge shelves are very obvious on the lateral margins of the longitudinal ridges (Fig. **6**) where they form conspicuous, parallel ledges. Each shelf is set at a slight upward angle (1°-3°) to the plane of the scale, and if traced distally, terminate in a scute or scaly process on the crest of the ridge. The scutes are imbricate and their apices project from under the apices of proximal scutes. Scutes are also prominent features of other scales (see Figs. **10, 12, 13**), but there are not as many shelves beneath them.

These shelves and the air spaces between them constitute a thin-film, interference-reflectance system. The precision with which the superimposed surfaces must be arranged in order to reflect certain wavelengths of light and the refractive index of the material in the shelves, determines the color reflected and its intensity. Ghiradella *et al* (1972: 1216) state that the average thickness of the lamellae in *Eurema lisa* is 550 ± 35 Angstroms, and the air spaces between shelves is 826± 42 Angstroms. From the known refractive index of air (1.0) and the inferred approximate refractive index of shelf cuticle (1.60, see discussion in chitin below), Ghiradella *et al* (*loc. cit.*) assumed that the shelves and the inter-shelf grooves had similar optical thicknesses (shelves: 880 ± 56 Angstroms, grooves 826 ± 42 Angstroms). Considering preparation distortions and measurement errors with the electron microscope, these authors were convinced that any disparity between the optical thicknesses of the two regions would be insignificant, and the common thickness of 858 ± 51 Angstroms assumed. They thus claimed that in *Eurema lisa*, the superimposed layers would function in the manner of a "quarter-wavelength interference reflection filter", which should reflect maximally at 343 ± nm. They confirmed this with reflection spectroscopy where the maximum ultraviolet reflection (corrected for tilt of the ridges on the longitudinal ridges) was 348 ± 2 nm.

During the present study we duplicated work on *Eurema*, and while our measurements on the scales were similar to those of Ghiradella *et al* (*loc. cit.*), sufficient significant differences were encountered to warrant further study. We anticipate more detailed investigation and separate publication on this question at an early date.

Since the ultraviolet reflectance of *Phoebis* and *Eurema* scales is produced by optical interference, the refractive index of the shelves of these ridges may vary depending on the presence or absence of chitin; the intervening intershelf grooves would also have to be correspondingly larger or smaller (depending on the refractive index of the shelf) in order for reflected light waves to reinforce rather than cancel one another.

This suggests that the inclusion of chitin or other chemicals in the plasticized shelf system needs also to be related to the structural arrangement in order to produce the consistency of color reflection/absorption usually noted in wing scales. In tests by Richards (1947) for chitin in butterfly wings, the alkaline treatment usually destroyed the iridescent qualities of scales. The iridescent scales of

Doxocopa (= *Chlorippe) seraphina* were an exception to this treatment, however, retaining their iridescence; this indicates a basic difference in the cause of the iridescence in this species, and perhaps others.

We were also able to observe intra-shelf sinuses inside the longitudinal ridges of *Phoebis*. There appeared to be seven or eight such cavities in each ridge. Since they follow the downward angle of the shelves, we assume they communicate with the air space in the lumen of the scale, near the point of junction of the shelf-line with the obverse surface. The scute on the crest of the ridge lacks the sinus towards its apex.

The occurrence of ridge sinuses indicates that the optical properties of the ridge system corresponds to those reported by Ghiradella *et al* (1972) in *Eurema*, and by Ghiradella (1974) in *Pieris*. It would appear that the gradually enlarging sinuses (moving dorso-ventrally in a transverse section, or from the distal apex of the sinus near the scute to its proximal union with the lumen) maintain a fairly uniform light refracting or reflecting qualities at any point on the ridge.

Beneath the lowest two or three shelves is the lumen of the scale, which projects upward, forming an inverted "V"-shaped cavity beneath each ridge.

The U-V reflecting scales in *Phoebis* always contain ovoid bodies (=pterinosomes) which can be noted between the ridges in Fig. **7.** They are attached to cross ribs and the trabecular braces beneath ridges. They may contribute to the visible colors noted in these scales as will be discussed below.

U-V reflectant scales with high longitudinal ridges were the cover scales in sample area 2. In addition, sample area 1 of *philea* and area 3 of *avellaneda* had cover scales of this type, as was predictable from the U-V photographs (Fig. **1a,** and **3a**). In all cases, basal scales in these areas were small (hidden), ladder-like, pigment bearing, yellow-type scales. The correlation of scale type with U-V properties was further demonstrated by SEM observations in other *Phoebis* species. *P. argante* (Fig. **5a**) had high-ridged scales in sample area 4, and proximal 5, as well as area 1 and 2.

Directional Reflectance. Various authors have called attention to the "on-off" directional reflectance of the ultraviolet in some pierids; oblique illumination from one side of the specimen causes only the scale-patch on the opposite side to "light-up". The wings or patch on the same side as the source of illumination absorbs, rather than reflects in the U-V wavelengths. These authors include: Nekrutenko (1965b) in *Gonepteryx rhamni* where the phenomenon was described as a "gynandromorphic" effect and a "dip angle"; Eisner *et al* (1969) photographed in *Phoebis rurina* and indicated for other genera and described as "directional iridescence"; Ghiradella *et al* (1972) in *Eurema lisa* and *Colias eurytheme;* and Ferris (1973) in *Colias alexandra.*

Of the species reported herein, only *Phoebis argante* and *A. statira* exhibited this phenomena. However, in corroboration of earlier reports, we have found numerous other species in other genera show this "blinking". Our preliminary studies tend to confirm the shelf-system angle produces this effect as reported by Ghiradella *et al* (1972) in *Eurema Lisa.* Our findings with respect to directional reflectance will be reported in detail in a following paper.

Not all male pierids exhibit U-V reflectant properties. The fact that *Aphrissa boisduvali* (C. Feld.), *Colias philodice* Godt. and *Phoebis sennae* (Linn.) lack this character, while their near relatives show scale reflectance, might permit some evolutionary inferences. Cover scales appear to be absent in all these species, and the remaining basal scales lack the high reflectant longitudinal ridges. The ultraviolet reflectance in females of *Colias chrysotheme* reported and shown in photographs by Silberglied and Taylor (1973) is very similar to the "mirror" reflectance obtained from wing membranes devoid of scales, or those whose cover layer of scales has been removed by rubbing.

Ultraviolet-reflectance is inherited as a sex-linked recessive trait in *Colias* (Silberglied and Taylor, 1973: 408). This was determined from examination of preserved specimens of Gerould's genetic broods at Yale University, and by experi-

mental crosses by Taylor (*op. cit.*). Ultraviolet absorbance is sex-linked dominant. The mode of inheritance of this trait in *Phoebis* is unknown.

Perforated-type scales (Figs. **12, 13**). This category of scale, which was distinguished by Suffert (1924) (=*Lochreihentypus*), has numerous "pores" or windows-on-the-septum between the longitudinal ridges. An equally distinctive feature is that of the transverse flutes. These are a continuation of the vertical or oblique flutes or foldings on the lateral margins of the longitudinal ridges. It is presumed these folds impart more strength to the surface from which they originate, but their linear, parallel nature makes them obvious features, and imparts a pinnulate or feather-like appearance to such a scale. This is particularly apparent in one grouping of this type of scale (Fig. **13**) in which the windows are reduced to small, pore-like slits, appearing in irregular positions on the septum. Such a scale has much more of a reflective obverse surface than the scale whose windows are large and rectangular, where ovoid bodies in the scale lumen can be readily observed. It is assumed that this "feather-like" scale type accounts for the minor mirror type U-V reflectance in the marginal and submarginal areas as may be noted in Fig. **1a, 2a** and **3a.**

The nature of the windows on this scale type enhances the disparate appearance of extremes, and emphasizes scale differences rather than similarities. This is demonstrated in comparing Figure **12** with Figure **13**. The former has fairly uniform, obtuse to rounded windows, with a degree of regularity to the width of the cross ribs between openings. In the latter, one might assume that windows are lacking, and that only an occasional opening or pore-like slit breaks the continuity of the surface. Occasional scales can be found which lack these openings. The ultrastructure of such different appearing scales is remarkably similar, and seems to vary only with the size of the window. In fact, they represent stages along a presumed continuum which ends with the fully "opened" windows as may be noted in the pigment scales discussed above. For purposes of calling attention to their possible increased mirror reflectance, we are here separating the fully-opened window condition, from all those with smaller openings, and an increased number of transverse flutes.

While the basic ultrastructure of this particular scale type is fairly uniform, the pattern of the longitudinal ridges and the sculpturing on the obverse membrane shows some variation from place to place on a single scale. That is, the basal area may show a transition between several conditions of window types, as shown in Figure **16**. Nearer the pedicel, the obverse membrane may lack openings, but in traversing an inter-ridge channel to more distal positions the windows are at first small pores, with much transverse fluting between, to irregular medium-sized, round opening exposing about half of the linear distance between ridges, to the fully opened rectangular windows observed in pigmented cover scales (above).

Another feature shared by most of these scale types is the occurrence of ovoid bodies in the scale lumen. They may be clear, yellow, orange, or black (Fig. **15**) should there be melanin deposited in the lamellar or other surfaces (see below).

Wing samples from areas 5, 6 of the forewing, and 11, 12 of the hindwing, contained these pinnulate scales. In these areas, pinulate scales with medium sized, irregular-shaped windows (Fig. **12**), tended to be in elongate scales with a rather acute apex (Fig. **11a**). The small-pored scale (Fig. **13**) whose inter-ridge septum appears composed largely of concentric transverse flutes (not infrequently mistaken for cross ribs in light microscopy studies) represents the surface sculpturing of shorter spatulate scales (Fig. **11b**) with obtuse apices and attenuate basal margins.

Black scales, transparent scales, and dentate yellow scales from area 5 resemble one another very closely in surface ultrastructure. The black scales lack the great quantity of ovoid bodies of the other two types, but the black scales do not differ (at least superficially) in other morphological features. One way to account for the color difference in the latter case is to assume the presence of melanin pigments in both the longitudinal ridges and obverse membranes and cross ribs of the black

scales. This would mask any yellow color which might be caused by pigments beneath. Visual proof of the occurrence of melanin in the ridges was obtained during this study by color photography of the marginal black scales in both *P. philea* and *P. thalestris*. At high magnification (200) the longitudinal ridges appeared as black stripes against the lighter inter-ridge channel. Yellow scales of the same size, shape and physical appearance except for color, occur in the same marginal areas; presumably the only fundamental difference is in the internal melanin deposits.

We cannot account for the transparent nature of some of these scales when they contain numerous "so-called" pigment bodies. They do not appear to otherwise differ from the yellow scales, and except for direct and careful observation, would have been thus classified by SEM appearance only. Clearly, careful chemical analysis is warranted.

Differing amounts and varying sites of melanin deposits within single scales have been reported in other pierids (Hirata & Uehara, 1959; Hirata & Kubota, 1957; Yagi, 1954, 1955; Hidaka & Okada, 1970).

We have not been able to confirm in *Phoebis* the possible occurrence of a second structural type of pigment body, or a type whose normal position is horizontal in the lumen lying against the underside lamella, as noted by Hidaka and Okada (1970) in *Pieris*. This included scale samples from the same relative position of the hindwing as well as many other locations. The occasional ovoid body observed in a horizontal position in the scale lumen was presumed to be accidentally dislodged from its normal position attached to the obverse cross ribs, under-ridge, supportive struts or trabeculae. This was confirmed in cases where scales were subjected to harsher handling (chemical extraction and/or scale incision and fragmentation) to obtain cross sectional views (see Fig. **17**) where greater numbers of ovoid bodies would be disturbed, and could be found in atypical positions.

Hopkins (1895) first proposed that the white and yellow pigments of the Pieridae were compounds related to uric acid and were restricted to members of this family. A host of other studies commencing with Wieland and Schopf (1925) and as recent as Lafont (1975) and Descimon (1976) have detailed the chemistry and relationships of the specific pigments involved. The complexity of the physiology of pigmentation has made it much more difficult to understand the biological role of pterins in wing pigments. However, it is generally acknowledged that they function in intra- and inter-specific signals (communication) and have excretory utility.

While there are a limited number of wing pterins (12 or less) which have been identified from various pierid wings, it has been assumed that most, if not all of these have been confined to the wing scales, and specifically to the pterinosomes, of "pigment" bodies. As indicated above, these bodies are round, elliptical or rod-shaped structures located in the lumen (inter-trabecular sinus) of the scale. They appear to be loosely attached to cross ribs as well as trabecular struts and braces, and hang suspended in the lumen with the longitudinal axis (if any) pointed downward as if by gravity. Other scale pigments, such as melanius and tryptophan derivatives (ommochromes, papiliochromes) may be located in other structural parts of the scale, which may hinder extraction by some methods.

CONCLUSIONS

Pronounced U-V reflectance patterns occur in males of most species in the genus *Phoebis*. The brilliant U-V iridescence on the male forewing basal and discal areas is produced structurally in the longitudinal ridges of the cover scales. This was confirmed by spectroscopy and scan electron microscopy. Lesser intensities of light in the 200-400 nm range, other than that produced in the longitudinal ridges, is primiarily caused by mirror reflectance. This is associated with the inter-ridge septum of scales of the perforated type, which are primarily marginal and sub-marginal in occurrence.

Not all species of *Phoebis* and relatives have males showing high intensity

U-V reflectance patches or patterns. In those males that show the trait, it is subject to less variation than color types (visible and U-V) which may occur in the females. We believe it is used as a sexual recognition signal.

A minimum of two types of scales occur in all wing samples taken: these may be grouped by a horizontal arrangement of the supine scales into covering scales and basal scales. In proximal regions of the wing (extending almost to submarginal areas) the sockets of covering and basal scales are arranged in alternate transverse rows which show various degrees of coalescence; in the marginal area, adjacent socket rows lie side by side forming an irregular, semi-tiered group of sockets. In some cases, the rows become superimposed, and the "single" row resulting will then be composed of alternate sockets of cover and basal scales. Sockets of "specialized" scales such as basal "hair" scales and marginal (border) black scales may be included along and between regular tiers as extras.

Submarginal and marginal areas in *Phoebis* have less distinct layering, and scales of intermediate position (the Mittelschuppen of Kuehn and Henke, 1929) are the rule; often they are indistinguishable in type from cover scales above or basal scales below, but admixtures of several scale types are not uncommon in this region.

Several ultrastructural details were noted in wing scales of the genus *Phoebis* for the first time:

1) Over twice as many longitudinal ridges per unit scale width occur on U-V reflectant cover scales as occur on basal (or regular) pigment-bearing scales. Ridges on the former are twice as high as the width of their inter-ridge channels; in basal or pigment-bearing scales in this genus, the longitudinal ridges have a low profile, which seldom finds them over 1/3 as high as the width between adjacent longitudinal ridges (measured crest to crest).
2) Ridge shelves on the lateral margins of the longitudinal ridges are in the opposite (rather than laternate) position in *Phoebis*. While there is some variation in shelf number even in the same scale, some species show significant differences (viz. *philea* shelf number 10-11; *argante* shelf number 6-7).
3) The absolute intensity of U-V reflectance in *Phoebis* males may vary slightly between individuals of the same species, but more significantly, between species. Some of the ultrastructural features observed which help account for this include: height and sinuous nature of the longitudinal ridges; number of longitudinal ridges per given width on the obverse surface; number of shelves per ridge.
4) Wing-size of the specimen (giant, dwarf or average) did not appear to influence U-V reflectivity; this verifies the relationship between reflectivity and ultrastructure established for this character.
5) Ridge sinuses are present, which together with ridge shelves in an apposite position indicate that the optical properties of the ridge system corresponds to those reported by Ghiradella *et al* (1972) in *Eurema*, and by Ghiradella (1974) in *Colias*.
6) The lumen of the scale forms an inverted "V" shape at the base of each longitudinal ridge, and extends vertically to occupy a position beneath the lowest 2 or 3 of the parallel ridge shelves. The intra-shelf sinuses occur between the upper 7 or 8 shelves; the apical portions of the scute (or scaly process) on the crest (summit) of the ridge usually lacks a sinus.
7) The U-V reflectant scales in *Phoebis* contain ovoid bodies, and show color characteristics in the visible spectrum (yellow, orange, red) not dissimilar from scales which absorb the U-V wavelengths.
8) Differences in pigment extractions of these scales and others suggests that at least two chemical types of (yellow-orange) pigment are involved and/or that they may be physically bound in a different manner within the scale, and should be studied further. Bleaching or extraction of these pigments does not change the U-V reflectance properties of the scale to a significant degree: such scales appear colorless against both white and black backgrounds.
9) Scales with ovoid bodies may be colored, or be black, or transparent. Evi-

dence was present to show that melanins occur in the longitudinal ridges, and elsewhere in the scale, and may mask other pigments within. The transparency of some scales bearing ovoid bodies is as yet unexplained.

10) Scale samples from various areas of the same wing had fewer ultrastructural differences than expected. Scales from some wing areas, *i.e.*, discal regions, had greater inter-specific diversity than did scale samples from other comparable wing areas of different taxa.

11) Male U-V reflectance is reported for the first time in the following *Phoebis* species or near relatives: *Aphrissa statira* (Cram.); *P. agarithe* (Bdv.); *P. avellaneda* (H.-Schaeff); *P. neocypris* (Hbn.); *P. thalestris* (Illiger); *Prestonia clarki* Schaus; *Rhabdodryas trite* (Linn). The males of *Aphrissa boisduvali* (C. Feld.) and *Phoebis sennae* (Linn.) lack marked U-V reflectant properties, which parallels the case in *Colias* where male *C. philodice* lack U-V reflecting scales while most other *Colias* species possess such characters. The variable occurrence and intensity of U-V reflecting scales in the female also attests to the lack of uniformity of this type of sexual dimorphism throughout the genus *Phoebis*.

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