STUDY OF FLUORESCENT PIGMENTS IN LEPIDOPTERA BY MEANS OF PAPER PARTITION CHROMATOGRAPHY[1]

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Subsequent to the extensive study of organic pigments in Lepidoptera by Ford (1941–1955), very little has been done to advance our knowledge of pigments in butterflies. There have been, however, some specialized investigations by a group of biochemists and geneticists interested in the chemical structure of pteridine compounds (Hadorn, 1962).

Having been interested in butterflies during my youth and as an avocation for over half a century, I have devoted a number of years after retirement in an attempt to continue research on organic pigments in butterflies by employing the comparatively new yet popular technique of paper partition chromatography. Based on studies of fluorescent pigments of many species, genera and families of butterflies on chromatograms, including the distribution of pigments in various parts of the body, I hope this paper will be of interest to fellow lepidopterists.

Sufficient evidence has been obtained to show that the pigments in Lepidoptera and other orders of insects, particularly the fluorescent pteridines, are correlated to morphological taxonomy and that this principle can be a valuable auxiliary aid in systematics.

HISTORICAL REVIEW

Apparently, the first person to study the chemistry of pigments in butterflies was Hopkins (1891, 1895 a, b, c). He discovered two water soluble pigments, leucopterin and xanthopterin, in the wings of white and yellow pierid butterflies, respectively. The chemical structure of these compounds, however, was not known until they were re-examined by Wieland and Schöpf.[3] They are regarded as purine compounds and are called pterins or pteridines, the name being derived from the Greek work for wing "pteron." Thirty years after Hopkins' papers, Cockayne (1924) made a study of reactions of butterflies' wings when examined

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[3] Darcy Gilmour, in his "Biochemistry of Insects," states that the above biochemists established the structure and synthesized xanthopterin in 1925 and shortly afterwards included the white pigment, leucopterin, as well as isoxanthopterin. However, he does not give a specific reference. The reference probably is: Wieland, H. and C. Schopf, 1925. Uber den gelben Flugelfarbstoff des citronenfalters (*Gonepteryx rhamni*). *Chem. berichte* 58:2178–2180.—[ASST. ED.]

under ultraviolet light. He revealed fluorescence in certain species and groups and investigated possible relationship between fluorescence and taxonomic position. He found that a significant degree of correlation did exist between fluroescent species and taxonomy. Cockayne's lead was followed 17 years later by Ford (1941, 1942, 1944, 1945, 1947 a, b), whose series of papers and publications represent the first comprehensive study of the nature, distribution and taxonomic importance of pigments in butterflies.

Ford's method of determining pigments consisted of exposing specimens to fumes of chemical reagents that temporarily changed their color. This method is not satisfactory for the detection of pigments other than those in superficial structures such as in the wings. Apparently, paper partition chromatography had not been sufficiently developed to be in general use at the time Ford's work was conducted.

Paper Partition Chromatography and Preparation of Chromatograms

Since a majority of lepidopterists may not be familiar with the subject of paper partition chromatography, the following discussion will introduce the subject. This should provide full particulars for those interested in paper partition chromatography, particularly as it applies to study and fractionation of organic pigments in Lepidoptera or other insects.

The first principles of chromatography were discovered by a Russian botanist, Michael Tswett in 1906. He fractionated the pigments of plants by pouring a mixture of petroleum ether and chlorophyll into a glass cylinder packed with calcium carbonate. As the solution percolated through the calcium carbonate, various components of chlorophyll were absorbed at different levels.

It was not until nearly forty years later that his technique became recognized as an exceptionally efficient method for the separation and identification of complex mixtures of gases, liquids, or dissolved solids. In recent years the method has attained a high degree of usefulness in both industry and science.

A modification of Tswett's method, using paper, was developed by Consden, Gordon and Martin (1944) and has attained great popularity because of its simplicity, rapidity and high resolving power. The writer is using the ascending modification of this method introduced by Williams and Kirby (1948), a description of which follows:

1. I use Pyrex battery jars (18″ × 6″) which make excellent containers for making both one way and two dimensional chromatograms.
2. To prepare a sheet of paper for use as a chromatogram I fold a sheet of commercially available Whatman No. 1 chemically prepared

filter paper measuring, approximally, 18″ × 22″ into halves (longitudinally). This gives two rectangular sheets 11″ × 18″ which is about the right size (when formed into a cylinder) to fit inside a battery jar six inches in diameter. A pencil line is then drawn one inch above and parallel to the 18″ margin. This is the "base line" on which tissue samples are to be placed. Another line should be drawn about 13″ above and parallel to the base line. This second line is intended to mark the place where the solvent ascends to and is called the "solvent front."

3. Insect tissue is prepared by placing specimens to be processed in a small agate or porcelain mortar together with a few drops of water, and ground to a fine paste about the consistency of thick cream. A small quantity of thoroughly ground paste (tissue sample) is applied on the base line at regular intervals of 25 mm.

4. After the tissue samples are dry the two shorter ends of the paper are brought together (to form a cylinder) and fastened with metal staples. Care should be taken to prevent the two ends of the paper from coming in contact as distortion of solvent flow will result if this occurs.

5. The paper cylinder is now ready to go into the battery jar, but first, a mixture of solvents should be added. For insect pigments, two solvent systems have proven satisfactory, *a*) propanol and one per cent of ammonium hydroxide, (in a ratio of 2 : 1), and *b*) butanol, distilled water and acetic acid, ratio (4 : 5 : 1). Approximately 50–75 ml of solvent is sufficient. After the paper cylinder, or chromatogram has been placed in the jar (the tissue sample end in contact with the solvent) a thick piece of plate glass is placed on top of the jar to prevent evaporation and to maintain a suitable degree of humidity inside the jar. At a temperature of 70°F. ± the flow of solvent should reach the solvent front, or finishing line, in 12 to 14 hours.

After the solvent front has been reached the chromatogram is removed and allowed to dry. When first removed from the jar, nothing is to be seen on the chromatogram paper. However, when exposed to ultraviolet light a fluorescent column appears, made up of tiers or divisions of different shades of color, each one of which is characteristic of individual components fractionated from the tissue samples by the solvent.

Fractionation of Soluble Pigments by Means of Paper Partition Chromatography

The process of fractionation has been theoretically explained by physicists and chemists, but as this is quite involved the following simplified attempt to explain what makes fractionation work may be more understandable.

As the solvent ascends the paper, by capillary attraction, it passes through tissue samples (spotted on the base line) and thence carries soluble components to various heights on the paper where they are absorbed. This is due to the varying molecular weights and ionic charges of each compound. The particular place of deposit is determind by the chemical nature of fractionated components and is termed the "Rf value" or ratio-to-front. This feature is symbolic because the particular color of fluorescence, together with the Rf value, is of diagnostic significance. On uni-dimensional ascending chromatograms, overlapping or fusion of fractionated pigments may occur. A two dimensional method corrects this difficulty. The fluorescent pattern of freshly prepared chromatograms fades quite rapidly. Therefore, in order to keep a permanent record for study it is necessary to preserve the image of this fluorescent pattern. Colored photographs for projection or enlargements may be made. However, as these are quite expensive, the writer has found it practical to trace the outlines of fluorescent areas and patterns with a soft pencil, while holding a chromatogram before an ultraviolet lamp. Following this, the marked outlines on the chromatogram are retraced on drawing paper. To make the copy as realistic as possible, I fill in, with water color paint, to approximate the fluorescent areas seen on the original chromatogram when exposed to ultraviolet light. With the title and other necessary data added, this replica is available for study and filing for future reference. All the illustrations in this paper were prepared from replicas made in this manner.

OCCURRENCE AND NATURE OF ORGANIC PIGMENTS IN LEPIDOPTERA

Organic pigments in butterflies and other insects consist of two kinds, pigments manufactured in the body as a result of metabolic processes, and those obtained from food consumed during the immature stages and passed on to the adult.

Pteridine pigments and some flavones, anthocyanins and other plant pigments are fluorescent on chromatograms when examined by ultraviolet light. However, melanin, ommachrome pigments, kynurenin, carotenes, and some others are not fluorescent pigments. For this reason they are not discussed further in this paper. Chemical structures of these compounds must be determined by special tests and chemical reagents.

Anthocyanins or Flavones

These are important plant pigments widely distributed in the petals of flowering plants. Adult butterflies acquire their flavones directly

from the food plants of the caterpillar. A classical example of this is the presence of flavones in the wings of the European marble white butterfly, *Melanargia galathea* Seitz, which gets its "marble white" pigment from coltsfoot grass, *Dactylis glomerata*, consumed by the larva. Ford (1945, 1946) found flavones in the wing scales of several unrelated English butterflies by the simple but effective process of exposing mounted specimens to the fumes of ammonium hydroxide which changes areas of the wings containing flavones to a bright yellow shade. The species Ford examined are *M. galathea*, *Coenonympha tullia*, *Erynnis tages*, and *Pyrgus malvae*. The present writer found flavone pigment in the wings of *Pyrgus communis* (Grote), which is similar to the European *P. malvae*. However, no experiments investigating the fluorescent nature of these compounds were conducted.

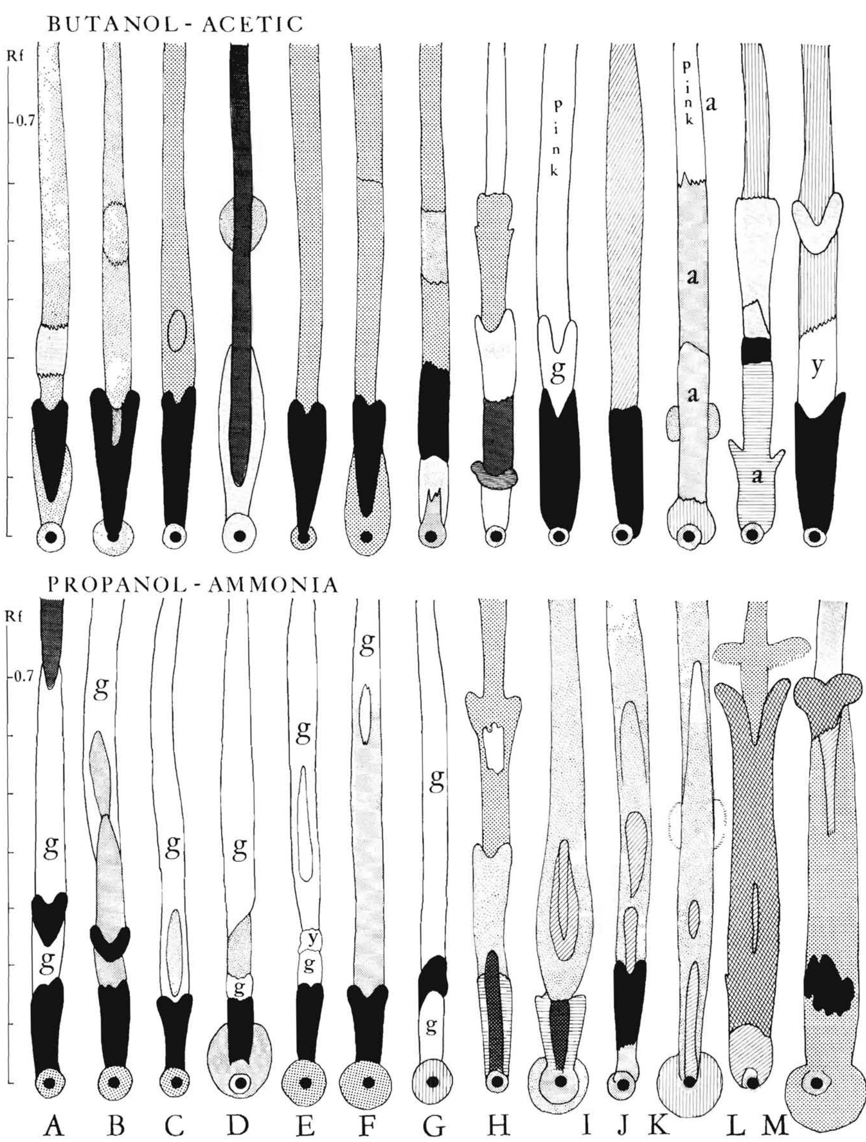
Some species of satyrids have flavones in the scales of their wings. Dos Passos (1948) was able to rearrange the taxonomic status of two groups of Nearctic *Oeneis*, namely, *O. uhleri* and *O. taygete* by exposing them to the fumes of ammonium hydroxide (28% NH_3), and he concluded that the chemistry of the pigment in the wings of Nearctic *Oeneis* assists greatly in their systematic arrangement.

Pteridines

One of the most outstanding characteristics of pteridine compounds is their fluorescence. In addition, they are considered to be the most important and widespread of insect pigments, particularly in the Lepidoptera (Wigglesworth, 1964). Chemically, they are purine compounds, and their skeleton structure was established by Purrmann in 1940 as a fused pyrimidine-pyrazine system.

The pteridines are closely related chemically to the flavones, some of which are also fluorescent. In many butterflies and moths pteridines play a special role in the wing scales by assuming patterns of color. In this respect they are prevalent in butterflies of the family Pieridae in which they were first discovered.

The pteridines known to occur in butterflies are leucopterin, erythropterin, and xanthopterin. Recently, Watt (1964) isolated the yellow fluorescing sepiapterin in the alfalfa butterfly, *Colias eurytheme* Bdv. This pteridine is photo-labile so that it may be overlooked unless chromatograms are processed in the dark. Another pteridine which is photo-labile is the blue fluorescing biopterin, which I believe occurs in Lepidoptera but which has been overlooked when chromatograms have not been developed in total darkness.



EXPLANATION OF PLATE 1

Replicas of unidimensional ascending paper chromatograms obtained from two different solvent systems which show presence of fluorescent compounds in the bodies of thirteen species of Lepidoptera. A. *Euptychia hermes sosybius* (F.), B. *Danaus gilippus berenice* (Cramer), C. *Heliconius charitonius* (L.), D. *Junonia coenia* (Hbn.), E. *Hemiargus ceraunus antibubastus* (Hbn.), F. *Papilio palamedes* (Drury), G. *Calephelis virginiensis* (G.-M.), H. *Colias eurytheme* (Bdv.), I. *Phoebis agarithe* (Bdv.), J. *Pyrgus communis* (Grote), K. *Panoquina panoquinoides* (Skinner), L. *Epistor lugubris* (L.), M. *Xanthopaster timais regnatrix* (Grote).

The most prevalent pteridine pigment in butterflies is the bright blue-violet fluorescing isoxanthopterin. Table 1 illustrates the prevalence or absence of this pteridine in three parts of the body of 67 species of North American butterflies.

While it may not be possible to recognize the specific chemical nature of some of the fluorescent pteridine components to be seen on chromatograms, their characteristic grouping (called the pteridine pattern) furnishes supportive evidence of the relationship of pigments to systematic taxonomy. It can be seen on Plate 1 that the pteridine pattern is distinctive of each species, even in intergeneric groups.

My experience has been that the pteridine pattern is not a very satisfactory guide for the differentiation of groups larger than genera, except in such a case as the family Pieridae.

Each of the replicas shown in plate 1 represent original chromatograms prepared with two kinds of solvents, namely No. 1, propanol and ammonium hydroxide and No. 2, butanol, acetic acid and water. Attention is called to this fact because each kind of solvent produces noticeable differences in the fluorescent pattern as well as in the position (Rf value) of fractionated components.

Isoxanthopterin in butterflies

Table 1 is a compilation of the presence or absence of the purple fluorescing pteridine, isoxanthopterin, in the head, body and wings of 67 species of North American butterflies. All the species listed were represented by males with two exceptions, *Speyeria diana* and *Appias drucilla neumoegenii.* In most cases, only a single specimen of each species listed was used to obtain the data recorded in this table, consequently a certain degree of latitude should be allowed for probable inaccuracies. However, the prevalence of isoxanthopterin, particularly, in organs such as the head, eyes and antennae of Lepidoptera and other

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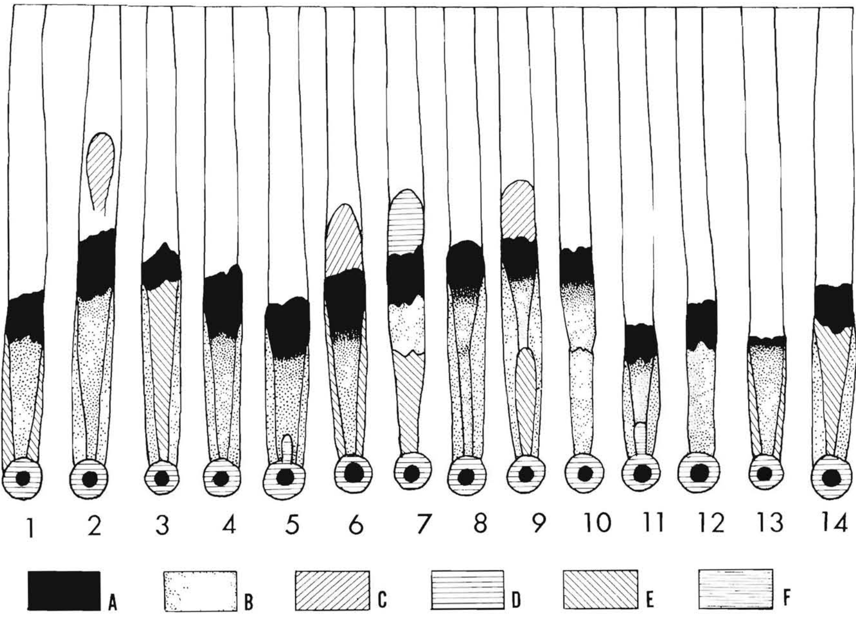
COLOR KEY TO PLATE 1

TABLE 1. DISTRIBUTION OF ISOXANTHOPTERIN IN BUTTERFLIES[1]

	HEAD	BODY	WINGS
Family PAPILIONIDAE			
Parnassius clodius (Menetries)	×	×	0
Battus polydamus (L.)	×	0	0
B. philenor (L.)	×	0	0
Graphium marcellus (Cramer)	×	×	(×)
Papilio glaucus glaucus L.	×	0	0
P. glaucus australis Maynard	×	0	×
P. polyxenes asterius Stoll	×	0	0
P. cresphontes Cramer	×	×	(×)
P. palamedes Drury	×	×	0
P. troilus troilus L.	×	0	0
P. troilus ilioneus J. E. Smith	×	×	0
Family PIERIDAE			
Colias interior interior Scudder	×	0	0
C. eurytheme eurytheme Bdv.	×	×	0
Eurema mexicana (Bdv.)	×	×	0
E. dina westwoodi (Bdv.)	×	×	0
Anteos maerula lacordairei (Bdv.)	(×)	×	0
A. clorinde nivifera Fruhstorfer	×	0	0
Phoebis philea (Johansson)	×	×	0
P. agarithe agarithe (Bdv.)	×	0	0
P. sennae eubule (L.)	×	×	0
Anthocaris midea Hübner	×	×	×
Pieris rapae (L.)	×	×	×
P. beckerii Edwards	×	×	×
Ascia monuste phileta (F.)	×	×	×
Appias drucilla neumoegenii (Skinner)	×	×	×
Appias drucilla neumoegenii (Skinner) female melanic form	×	×	×
Family DANAIDAE			
Danaus plexippus plexippus (L.)	×	×	0
D. gilippus berenice (Cramer)	×	0	0
Family SATYRIDAE			
Lethe eurydice (Johansson)	×	×	0
Euptychia cymela cymela (Cramer)	×	0	0
E. cymela viola (Maynard)	×	0	0
E. areolata areolata (J. E. Smith)	×	(×)	0
Oeneis polixenes katahdin (Newcomb)	×	0	0
Family NYMPHALIDAE			
Heliconius charitonius (L.)	×	×	×
H. petiveranus Doubleday	×	×	×
Agraulis vanillae nigrior Michener	×	×	(×)

[1] Symbols indicate relative quantities: × = average; (×) = less than average; 0 = absent.

	HEAD	BODY	WINGS
Family NYMPHALIDAE (*Continued*)			
Speyeria aphrodite alcestis (Edwards)	×	×	×
S. cybele cybele (F.)	×	×	×
S. diana (Cramer) male	×	×	0
S. diana (Cramer) female	×	×	×
Phyciodes tharos tharos (Drury)	×	×	×
P. batesii (Reakirt)	×	×	×
P. gorgone (Hbn.)	×	×	×
P. mylitta mylitta (Edwards)	×	×	×
P. frisia frisia (Poey)	×	×	×
P. campestris campestris (Behr)	×	×	×
Polygonia comma (Harris)	×	0	0
Vanessa atalanta (L.)	×	×	×
V. virginiensis (Drury)	×	0	0
Junonia coenia coenia (Hbn.)	×	×	×
J. evarete zonalis (C. & R. Felder)	×	×	×
Eunica tatila tatilista Kaye	×	×	(×)
Asterocampa clyton flora (Edwards)	×	0	0
A. celtis celtis (Bdv. and LeC.)	×	0	0
A. celtis alicia (Edwards)	×	0	0
Family LIBYTHEIDAE			
Libytheana bachmanii bachmanii (Kirkland)	×	×	0
L. bachmanii larvata (Strecker)	×	×	0
Family LYCAENIDAE			
Eumaeus atala florida (Röber)	×	×	×
Atlides halesus halesus (Cramer)	×	×	×
Euristrymon favonius (J. E. Smith)	×	0	0
Strymon melinus melinus Hübner	×	×	(×)
Everes comyntas comyntas (Godart)	×	×	×
Hemiargus ceraunus antibubastus Hbn.	×	×	0
Brephidium isophthalma pseudofea (Morrison)	×	×	×
Family RIODINIDAE			
Apodema nais (Edwards)	×	×	0
Lephelisca muticum (McAlpine)	×	×	0
L. borealis (Grote & Robinson)	×	×	×
Family HESPERIIDAE			
Phocides pigmalion okeechobee (Worthington)	×	0	0
Thorybes bathyllus (J. E. Smith)	×	×	0
Urbanus dorantes (Stoll)	×	0	0
Hylephila phyleus (Drury)	(×)	0	0
Percent of total	100.	61.	43.



EXPLANATION OF PLATE 2

Replicas of unidimensional ascending paper chromatograms which show presence of pteridines in bodies of fourteen species of butterflies (1–14) belonging to the genus *Phyciodes* and near relatives. Symbols A-F represent the different pteridines demonstrated.

orders of insects, seems to imply that this pteridine component plays an important role in metabolic activity.

Characteristic Pattern of Pteridine Pigments in the Genus Phyciodes and Closely Allied Genera

The purpose of this study was to determine whether species within a genus could be individually differentiated by the character of their pteridine pattern. In order to make sure that the pteridine pattern of a species was a standard one which could be used in comparison between species, a number of specimens of the same species and sex were employed in preparing chromatograms so that a specimen, showing the most characteristic pteridine pattern of a series, could be selected as typical for the species.

The replicas of fourteen species presented in plate 2 may be considered as representing the characteristic pteridine pattern of each species. It will be noted that none of the pteridine patterns are exactly

alike, and none reveal any common pattern or distinction which might characterize a genus. It may be noted, however, that four of the species, 3, 4, 5 and 11, resemble each other quite closely, although they represent different genera or subgenera. It might be inferred by this that their chemical make-up of these compounds shows more similarity than do their taxonomic relationships.

I think there is sufficient evidence in plate 2 to show that with the standardization and consistency of the pteridine pattern for comparative use it is possible to differentiate species within a genus. Caution must be excerised in drawing conclusions, however, because differentiation occurs also in infraspecific catagories such as in varieties, mutations, etc. For the determination of mutations in fruit flies (*Drosophila*) by means of paper partition chromatography, see Hadorn (1962).

A number of years ago the status of *Phyciodes tharos* (Drury) and *P. batesii* (Reakirt) was a controversial subject. Some authorities believed that *P. batesii* might be a seasonal form of *P. tharos* while others considered the former to be a separate species. In plate 2 it can be seen that the pteridine pattern of *P. tharos* (No. 4) and that of *P. batesii* (No. 6) are distinct. This helps confirm the contemporary taxonomic status of *batesii* by what might be called "chemotaxonomic testimony or supportive chemical evidence."

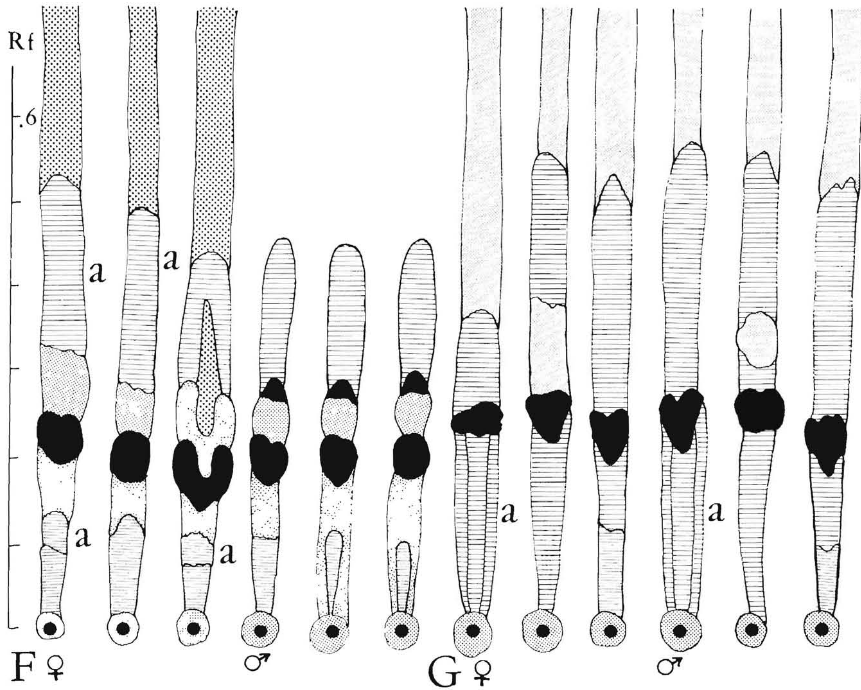
Pteridine pattern of pigments in green geometrid moths

Monochrome replicas of the pteridine pattern of two green geometrid moths (Subfamily Geometrinae), *Racheospila gerularia* (Hbn.) and *Synchlora denticularia* (Wlk.) are presented on plate 3. These show structural differences in the pteridine pattern of both species. While the chemical identity of all the fractionated pigments were not determined, the dark areas, centrally located in the vertical columns of both species, are undoubtedly the ubiquitous pteridine, isoxanthopterin. The pale columns above isoxanthopterin may be xanthopterin, which is relatively uncommon in butterflies other than the Pieridae. The short, pale basal areas (a) may be the xanthopterin of Good and Johnson (1949).

However, the principal point is not the chemical nature of the pigments themselves so much as the character of the fluorescent pigments which shows the relationship of biochemistry to structural morphology and the differentiation of these two species of moths.

Changes of pteridine pattern in the development of an amatid moth

The egg, larva, pupa, and adult of the polka-dot wasp moth, *Syntomeida epilais* (Wlk.), were examined for possible changes during



EXPLANATION OF PLATE 3

Replicas of unidimensional ascending paper chromatograms which show presence of pteridines in bodies of two green geometrid moths. F. *Racheospila gerularia* (Hbn.), G. *Synchlora denticularia* (Wlk.).

its metamorphosis. Experiments with two dimensional paper chromatography showed there are changes in the proportions of the various pteridines from one developmental stage to another.

SUMMARY

This paper is an introduction to the study of fluorescent pigments in Lepidoptera, based on observations made on the fluorescent pigments of a limited number of species of Nearctic butterflies by means of paper partition chromatography. Such aspects as chemistry, distribution in various parts of the body and the relation of pigments to the taxonomic status of species are stressed.

Observations have been made, by the single ascending and two dimensional methods of paper partition chromatography, on well over one hundred species of North American butterflies and a few species of moths. Because of the fleeting nature of fluorescent pigments on freshly

prepared chromatograms, it has been necessary to make carbon copies or colored replicas of all chromatograms in order to have a permanent record for study and for reference filing.

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NOTES ON *EUCHLOE AUSONIDES MAYI* (PIERIDAE) IN ONTARIO

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Syme (1961) reported about the occurrence of *Euchloe ausonides mayi* Chermock & Chermock in Ontario. From his report it can be seen that this species was first taken in Ontario in Malachi (Kenora District, near the Manitoba border) on July 5, 1947, by a summer field party of the Royal Ontario Museum. In 1956 Paim collected the same species on June 1, at Basswood Lake (Quetico Provincial Park, Rainy River District, near the Minnesota border).

To our knowledge of the distribution of *Euchloe ausonides mayi* in Ontario can be added the captures by Syme and Wood along the roadside of Highway 11, 3 to 15 miles east of Beardmore (Thunder Bay

[1] Paper read at the 103rd Annual Meeting of the Ent. Soc. of Ontario, Nov. 3, 1966, in Toronto, Ont.

segments I–VIII, and much larger dark brown pinaculi surround both dorsolateral spines on these segments. A narrow dorsal line from thoracic segment 2 to abdominal segment VIII. Thoracic segments 2–3 with dark brown pinaculi around upper dorsolateral spine. A heavy line connecting subdorsal dark pinaculi from thoracic segment 2 to abdominal segment VIII. Ventral surface light brown; boundary between brown and ochre yellow occurring between the upper and lower rows of subspiracular spines. Ventral surface, especially prolegs, covered with small reddish brown setae and hundreds of smaller transparent setae. Arrangement of spines (scoli) as in Figure 1. Setae of the most ventral spine in abdominal segments I, II, and VII unpigmented. Each large spine on dorsal half of body covered with about 20 minute setae, the longest (about 1.3 mm) at base and shortest at distal end of spine. Shorter spines with fewer setae. Leg with black trochanter and tarsal claw, other segments reddish brown. Ventral surface of legs covered with setae. Crochets biordinal, forming a lateral penellipse. Anal plate shown in Figure 2. Anterior lobe of anal plate dark brown, remainder reddish brown. Head reddish brown. Adfrontal sutures darker, separated from rest of head by pale sutures (Fig. 4). Ocelli and ocellar setae shown in Figure 3. Head with many dorsal and lateral setae; only those which have a constant position shown in Figure 4. Larvae began wandering on June 24; most pupated the following day.

PUPA: Length 15 mm. White, mottled with black stripes and spots as in Figures 5–7. Degree of melanism variable; in one individual many black areas were broken into separate spots, presenting a lighter appearance. Light brown showing faintly on dorsal surface: between black spots that are close together; in grooves between segments of abdomen (especially the grooves posterior to wing cases and one groove anterior to these grooves); and outlining wing cases. Light brown not showing on dorsum in a median one mm-wide strip except a few days before eclosure, when the segments posterior to the wing cases turn reddish brown. Ventral surface with light brown in the small spaces between the black in the space between the wing cases. Pupal stage lasts about eight days.

ADDENDUM

In the article "Study of fluorescent pigments in Lepidoptera by means of paper partition chromatography" by George W. Rawson (*J. Lepid. Soc.*, 22 (1): 27–40, 1968), the following additions and corrections should be made.

On page 31, the author of *Melanargia galathea* is Linnaeus, not Seitz.

On page 36, the names of the 14 *Phyciodes* and allies were omitted in the explanation of Plate 2. These are as follows: 1) *Chlosyne janais* (Drury); 2) *C. californica* (Wright); 3) *Phyciodes (Eresia) claudina guatemalena* Bates; 4) *P. (Phyciodes) tharos tharos* (Drury) form *"marcia"* Edw.; 5) *P. (P.) t. tharos* form *"morpheus"* F.; 6) *P. (P.) batesii* (Reakirt); 7) *Chlosyne i. ismeria* (Bdv. & LeC.); 8) *P. (P.) mylitta* (Edwards); 9) *P. (P.) campestris* (Behr); 10) *P. (Tritanassa) ptolyca* (Bates); 11) *P. (Eresia)frisia* (Poey); 12) *P. (Tritanassa) myia* (Hewitson); 13) *P. (Eresia) phillyra* (Hewitson); 14) *P. (Tritanassa) texana* (Edwards).

The color representation of the boxed symbols, A–F, accompanying this plate is as follows: A) Bright violet fluorescence; B) dull blue-violet; C) pale yellow; D) pale blue; E) grayish green; F) pinkish (in the basal portion of nos. 5 and 11).