

Distribution of Papiliochrome in Papilionid Butterflies

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Abstract Yellow, reddish brown, red, black, and white scales of the wings of thirteen papilionid species were examined for the presence or absence of Papiliochrome with thin-layer chromatography.

Pale yellow scales of *Papilio xuthus*, *P. demoleus*, *P. protenor*, *P. helenus*, *P. castor*, *P. polytes*, and *P. dardanus* have proved to contain Papiliochrome IIa and IIb as the major pigments. Besides, Papiliochrome IIIa and IIIb were found.

Deep yellow scales of *P. machaon* have proved to contain Papiliochrome M₁ and M₂ as the major pigments. Besides, the IIa, IIb, IIIa, and IIIb were found. The reddish brown scales of this species have proved to contain small quantities of IIa and IIb.

Yellow scales of *Lühdorfia japonica* have proved to contain the IIa, IIb, IIIa, IIIb, M₁, and M₂. Red scales of this species also showed very small quantities of IIa and IIb. Yellow scales of *Sericinus telamon* gave small quantities of IIa and IIb.

Black and white scales have proved to lack Papiliochrome.

No Papiliochrome was found in the scales of *Byasa alcinous*, *Menelaides aristolochiae*, and *Iphiclidides eurous*.

These results are discussed in connection with some earlier reports on the yellow pigments of Papilionidae.

Introduction

It is well known that the wing pigments of pierid and nymphalid butterflies are pterin and ommochrome, respectively. But the yellow pigments of papilionid butterflies are neither pterin nor ommochrome.

Since 1954, Umebachi and his collaborators have investigated the pale yellow pigments of the wings of *Papilio xuthus* and named them Papiliochrome (Umebachi, 1961, 1962, 1975a; Umebachi and Takahashi, 1956; Umebachi and Yoshida, 1970). Chemical and physical properties of the main yellow pigment, Papiliochrome II, of this species have been investigated especially in detail, and the pigment has proved to be formed from kynurenine and a N-(β -alanyl) DOPamine derivative (Umebachi, 1975a). In addition to Papiliochrome II, Papiliochrome III is present as a minor yellow pigment. Papiliochrome II and III are respectively separated into two components a and b, that

is, IIa and IIb or IIIa and IIIb. These yellow pigments readily decompose to kynurenine and the N-(β -alanyl) DOPAmine derivative, SN-1 (Umebachi and Yamashita, 1976, 1977).

The deep yellow pigments of *Papilio machaon* have also been investigated to some extent. These deep yellow scales contain the brownish yellow pigments M₁ and M₂ in addition to Papiliochrome IIa, IIb, IIIa, and IIIb (Umebachi, 1977a). The M₁ and M₂ have also been presumed to belong to the Papiliochrome group, because they are related to kynurenine, β -alanine, and an *o*-diphenolic substance.

The present paper gives some more data on the distribution of these six kinds of Papiliochrome (IIa, IIb, IIIa, IIIb, M₁, and M₂) in papilionid butterflies.

Materials and Methods

Materials

The thirteen species of Papilionidae listed in Table 1 were examined. All the butterflies used, except *P. xuthus* and *L. japonica* which were collected in Kanazawa, were obtained through the Okura Biological Institute. In all the species except *L. japonica* which was used without distinction of sex, male butterflies were used. Yellow, reddish brown, red, black, or white scales of the wings were scraped and stored.

Extraction and separation of pigments

Scales were first treated with 70% ethanol at room temperature. The extract (A) was, without any treatment, submitted to two-dimensional thin-layer chromatography, by which the presence or absence of Papiliochrome IIa, IIb, IIIa, and IIIb could be decided.

After the extract A was obtained, the scales were further treated with 70% ethanol at 40°C and then with 4% HCl-methanol at room temperature. The extract with 4% HCl-methanol was evaporated to dryness under reduced pressure, dissolved in water (Extract B), and submitted to two-dimensional thin-layer chromatography, by which the presence of M₁ and M₂ pigments could be confirmed.

Thin-layer chromatography

Cellulose thin-layer sheet (Merck, No. 5552; 20×20cm) was used. The solvent for the first dimension was 70% methanol (MeOH), and for the second dimension, a mixture of *n*-butanol-glacial acetic acid-water (120:30:50) (BAW).

After development, the chromatogram was inspected under ultraviolet light, and then either the ninhydrin test or the phosphomolybdic acid-NH₃ test (Umebachi and Yoshida, 1970) was performed.

Papiliochrome IIa, IIb, IIIa, and IIIb give yellow fluorescence under ultraviolet light and are yellowish brown to the ninhydrin test and blue to the phosphomolybdic acid-NH₃ test (Fig. 1a).

Table 1. Species and their scales of the papilionid butterflies examined

Family	Subfamily	Tribe	Species	Scales examined
		Papilionidae	Papilioninae	Papilionini
Black scales				
<i>Papilio demoleus</i>	Pale yellow scales			
	Reddish brown scales in the anal angle of hind-wings			
<i>Papilio protenor</i>	Pale yellow scales			
	Black scales			
<i>Papilio helenus</i>	Pale yellow scales on the upperside of hind-wings			
	White scales on the underside of hind-wings			
<i>Papilio castor</i>	Pale yellow scales on the upperside of hind-wings			
	White scales on the underside of hind-wings			
<i>Papilio polytes</i>	Pale yellow scales on the upperside of hind-wings			
<i>Papilio dardanus</i>	Pale yellow scales			
<i>Papilio machaon</i>	Deep yellow scales			
	Reddish brown scales in the anal angle of hind-wings			
	Black scales			
Gr- phiini	<i>Iphiclides eurous</i> (<i>Graphium</i>)	Yellowish white scales		
Troidini	<i>Byasa alcinous</i> (<i>Parides</i>)	Red scales		
		Black scales		
	<i>Menelaides aristolochiae</i> (<i>Pachliopta</i>)	Red scales		
Zerynthiinae	<i>Luhdorfia japonica</i>	Yellow scales		
		Red scales		
		Black scales		
	<i>Sericinus telamon</i>	Yellow scales		

The above-mentioned solvent systems were not suited for the M_1 and M_2 , because these two pigments remained near the origin of the chromatogram. But the presence or absence of M_1 and M_2 could be decided (Fig. 1b). These two brownish yellow pigments are blue to the phosphomolybdic acid- NH_3 test and, to the ninhydrin test, negative or slightly yellowish brown.

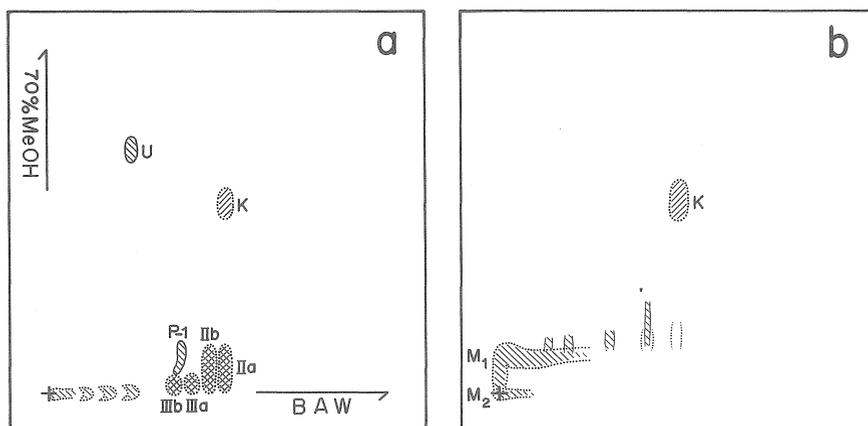


Fig 1. Two-dimensional chromatograms of (a) the extract A from the pale yellow scales of *P. xuthus* and (b) the extract B from the deep yellow scales of *P. machaon*.

Dotted circle, fluorescent substances; /// , ninhydrin-positive substances; |||| , substances positive to the phosphomolybdic acid- NH_3 test.

Spot K, kynurenine; IIa, IIb, IIIa, and IIIb, Papiliochrome; M_1 and M_2 , brownish yellow pigments; spots U and P-1 correspond to the spots U and P of the previous paper (Umebachi and Yoshida, 1970).

Results

The extract A of yellow scales

Two-dimensional thin-layer chromatogram of the extract A from the pale yellow scales of *P. xuthus* was already reported in the previous paper (Umebachi, 1977a), which showed that Papiliochrome IIa and IIb are the major pigments in this species and that the IIIa and IIIb are present as the minor yellow pigments.

The extract A of the yellow scales of *P. demoleus*, *P. protenor*, *P. helenus*, *P. castor*, *P. polytes*, and *P. dardanus* also gave essentially the same chromatogram as in *P. xuthus* (Fig. 1a). This indicates that the yellow pigments of these six *Papilio* species are also Papiliochrome IIa, IIb, IIIa, and IIIb (Table 2).

In the above seven *Papilio* species, the scales remaining after the repeated treatment with 70% ethanol at 40°C were only slightly yellowish.

Two-dimensional chromatogram of the extract A from the deep yellow scales of *P. machaon* also showed the presence of Papiliochrome IIa, IIb, IIIa, and IIIb as already reported in the previous paper (Umebachi, 1977a). But, the scales remaining after the repeated treatment with 70% ethanol at 40°C were still deep yellow. This indicates that the IIa, IIb, IIIa, and IIIb are not the main pigments of the deep yellow scales of this species.

As to *L. japonica*, the yellow scales have proved to contain Papiliochrome IIa, IIb, IIIa, and IIIb (Umebachi, 1977b). The yellow scales of *S. telamon* also showed the

Table 2. Distribution of Papiliochrome in the papilionid butterflies

Species	Scale	Papiliochrome
<i>P. xuthus</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
	Black	None
<i>P. demoleus</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
	Reddish brown	None; (R ₁)*
<i>P. protenor</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
	Black	None
<i>P. helenus</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
	White	None
<i>P. castor</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
	White	None
<i>P. polytes</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
<i>P. dardanus</i>	Pale yellow	IIa and IIb (Major); IIIa and IIIb (Minor)
<i>P. machaon</i>	Deep yellow	M ₁ and M ₂ (Major) IIa, IIb, IIIa, and IIIb (Minor)
	Reddish brown	IIa and IIb (small quantities); (R ₁)*
	Black	None
<i>I. eurous</i>	Yellowish white	None
<i>B. alcinous</i>	Red	None; (R ₂)**
	Black	None
<i>M. aristolochiae</i>	Red	None; (R ₂)**
<i>L. japonica</i>	Yellow	IIa, IIb, IIIa, IIIb, M ₁ , and M ₂
	Red	IIa and IIb (small quantities); (R ₂)**
	Black	None
<i>S. telamon</i>	Yellow	IIa and IIb

* Reddish brown pigment R₁

** Red pigment R₂. This does not belong to the Papiliochrome group.

presence of IIa and IIb (Tabl 2). In these two species, the yellow scales after the repeated treatment with 70% ethanol were still yellowish.

The extract B of yellow scales

In two-dimensional chromatogram of the extract B from the pale yellow scales of *P. xuthus*, *P. demoleus*, *P. protenor*, *P. helenus*, *P. castor*, *P. polytes*, and *P. dardanus*, the brownish yellow pigments, M₁ and M₂, were not found or, if any present, in a small or trace amount.

On the contrary, in *P. machon*, as already reported in the previous paper (Umebachi, 1977a), two-dimensional chromatogram of the extract B showed the presence of M₁ and

M₂. It was sure that these brownish yellow pigments are responsible for the color of deep yellow scales of this species.

The extract B of *L. japonica* has also proved to contain the M₁ and M₂ (Table 2). But, in *S. telamon*, the presence or absence of the M₁ and M₂ has remained unsettled, because the quantity of pigments was small and because only a limited number of samples was available.

The extract A of reddish brown and red scales

Two-dimensional chromatogram of the extract A from the reddish brown scales of the anal angle of *P. machaon* showed the presence of Papiliochrome IIa and IIb, though the quantities were small (Table 2). On the other hand, the reddish brown scales of the anal angle of *P. demoleus* did not contain the IIa and IIb. The reddish brown pigment of these two species could not be extracted by the repeated treatment with 70% ethanol (at 40°C) and 4% HCl-methanol (at room temperature) and remained insoluble in the scales.

The chromatogram of the extract A from the red scales of hind-wings of *L. japonica* also showed the presence of Papiliochrome IIa and IIb, though their quantities were very small. The red pigment was partly soluble in 70% ethanol, and the extract A was red. But the red pigment does not belong to the Papiliochrome group, because the pigment is not related to kynurenine.

The extract A of black and white scales

The black scales of *P. xuthus*, *P. protenor*, *P. machaon*, and *L. japonica* were examined in the same way as in yellow scales. In all the cases, Papiliochrome was not found at all.

The white scales on the underside of the hind-wings of *P. helenus* and *P. castor* were also examined in the same way as above, with the result that Papiliochrome was not found at all.

The absence of Papiliochrome in the Graphiini and Troidini

In the yellowish white scales of *I. eurous*, Papiliochrome was not found.

The red scales of *B. alcinous* and *M. aristolochiae* did not show Papiliochrome, either.

Discussion

In 1954, Umebachi and Nakamura reported that the hot water extract of the wings of some papilionid butterflies (*P. xuthus*, *P. protenor* (♂), *P. helenus*, *P. machaon*, and *L. japonica*) contains a large quantity of kynurenine. All these species have yellow scales in the wings. Since then, Umebachi and his collaborators (Umebachi, 1958, 1961, 1962, 1975a; Umebachi and Yoshida, 1970) have investigated physical and chemical properties

of the yellow pigments of these yellow scales, mainly using *P. xuthus*. It has proved that the yellow pigments are neither pterin nor ommochrome but the pigments which are related to both kynurenine and a DOPamine derivative. These yellow pigments were named Papiliochrome, which was a new group of insect pigments.

In *P. xuthus*, four kinds of Papiliochrome IIa, IIb, IIIa, and IIIb can be separated with paper or cellulose thin-layer chromatography. Among them, the IIa and IIb are the major yellow pigments of this species. The IIa and IIb readily decompose to kynurenine and the DOPamine derivative, SN-1, by being heated. The latter compound has proved to be a N-(β -alanyl)DOPamine derivative (Umebachi, 1975b; Umebachi and Yamashita, 1976, 1977). Moreover, Papiliochrome IIa and IIb have been presumed to be optically isomeric with each other (Umebachi and Yoshida, 1970).

In *P. machaon*, the brownish yellow pigments M₁ and M₂ were found in addition to Papiliochrome II and III. The M₁ and M₂ have been presumed to belong to Papiliochrome group, because they also release kynurenine, β -alanine, and an *o*-diphenolic substance on hydrolysis (1977a).

The present paper shows that, in addition to *P. xuthus* and *P. machaon*, the pale yellow scales of *P. demoleus*, *P. protenor*(♂), *P. helenus*, *P. castor*, *P. polytes*, and *P. dardanus* (♂) also contain Papiliochrome IIa, IIb, IIIa, and IIIb. It is probable that the pale yellow scales of the wings of other *Papilio* species also contain the IIa, IIb, IIIa, and IIIb and that the deep yellow scales contain the M₁ and M₂ in addition to the II and III.

The present paper furthermore shows that the yellow scales of *L. japonica* contain Papiliochrome IIa, IIb, IIIa, IIIb, M₁, and M₂. It is probable that the yellow scales of other species of Zerynthiinae also contain Papiliochrome. Umebachi (1959) reported that the hot water extracts of the wings of *Zerynthia polyxena*, *Z. rumina*, *L. japonica*, *L. puziloi*, *Bhutanitis lidderdalei*, and *S. telamon* contain kynurenine.

Cockayne reported in 1924 that the pale yellow markings of the wings of *Papilio* species give a bright fluorescence under ultraviolet light and that the dark yellow markings of the wings of *P. machaon* are almost dull under ultraviolet light. Now, from the results of the present paper, the bright fluorescence is probably due to Papiliochrome II and III and a small quantity of free kynurenine. On the other hand, the dull areas of *P. machaon* seem to be due to Papiliochrome M₁ and M₂.

Ford (1941) investigated the distribution of anthoxanthins in the wings of butterflies from the standpoint of systematics. According to him, among the Papilionidae, some genera contain anthoxanthins in their wings and others do not have them. *Graphium* is one of the former genera, while *Papilio* belongs to the latter. Interestingly, when Ford (1941) examined ninety two species of *Graphium*, he found that eighty two species contained anthoxanthins in their wings, while the remaining ten species did not show anthoxanthin and gave a yellow fluorescence. These fluorescences had already been reported by Cockayne (1924). Umebachi (1960) examined eighteen species of *Graphium* and found that the hot water extract of wings of the species which Ford described as possessing no anthoxanthin and as giving fluorescence contained kynurenine, while the

species which Ford described as possessing anthoxanthin and as giving no fluorescence did not contain kynurenine. It is probable that the fluorescence which Ford reported is due to Papiliochrome and a small quantity of free kynurenine. *I. eurous*, which did not show Papiliochrome in the present paper, is one of the species which were reported by Ford as possessing anthoxanthin in the wings and as giving no fluorescence.

In the Third International Pteridine Symposium, Schöpf (1964) mentioned that pigments of a new type might be found in papilionid butterflies and that their pale yellow is not due to xanthopterin. Papiliochromes which have been described in the present paper must be the very pigment group.

It is interesting that the reddish brown scales of the anal angle of *P. machaon* and the red scales of *L. japonica* contain small quantities of Papiliochrome IIa and IIb, in addition to the reddish brown or red pigment. These reddish brown and red pigments are tentatively named R₁ and R, respectively, in the present paper. Their chemical properties are now being investigated.

It is also interesting that Papiliochromes are not found in black scales even if the butterfly belongs to the genus, *Papilio*. There are some reports that the pupal cuticle of black mutants of some insects lacks β -alanine, while that of wild type contains this amino acid (Seki, 1962; Fukushi and Seki, 1965; Jacobs and Brubaker, 1963). The formation of N-(β -alanyl) DOPamine derivative may be one of the mechanisms by which melanin formation is depressed.

Furthermore, the presence of β -alanine in hardened cuticle has been well known (for example, Hackman and Goldberg, 1977). But the nature of bonding of β -alanine in cuticles has remained unsettled. Now, the occurrence of N-(β -alanyl) DOPamine derivative may give a clue to the problem.

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