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The functional morphology of color changing in a spider: development of ommochrome pigment granules

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SUMMARY

Studies on the formation of ommochrome pigment granules are very few, despite their generalized occurrence as screening pigments in insect eyes. This is particularly true for ommochrome granules responsible for epidermal coloration. The aims of this study were to characterize the localization of major body pigments in a color changing mimetic spider, *Misumena vatia* (Thomisidae), and to describe the formation and location of ommochrome pigment granules responsible for the spider's color change from white to yellow. The unpigmented cuticula of this spider is transparent. Both the guanine localized in guanine cells in the opisthosoma and the uric acid localized in epidermis cells in the prosoma are responsible for the white coloration. The bright yellow color is due to the combination of ommochrome pigment granules and the white reflectance from coincident guanine and/or uric acid. The formation of ommochrome pigment granules in epidermis cells proceeds *via* three distinctive steps. Translucent, UV fluorescent, progranules (*type I*) are produced by a dense network of endoplasmic reticulum associated with numerous mitochondria and glycogen rosettes. These progranules are present in white spiders only, and regularly distributed in the cytoplasm. The merging of several progranules of *type I* into a transient state (progranule *type II*) leads to the formation of granules (*type III*) characterized by their lack of fluorescence, their spherical sections and their osmophilic-electron-dense contents. They are found in yellow spiders and in the red stripes on the body sides. Their color varies from yellow to red. Thus, white spiders contain only *type I* granules, yellow tinted spiders contain *type II* and *III* granules and bright yellow spiders contain only *type III* granules. We present a synthetic view of the ontogeny of ommochrome granules. We discuss the physiology of color changing and the nature of the chemical compounds in the different types of granules. Extended studies on the ultrastructural modification and physiological processes associated with color change are required before any statement about the adaptiveness of the color change can be made.

Key words: animal color, epidermis, zoochromes, kynurenine, 3-OH-kynurenine, mimetism, crab-spider, *Misumena vatia*.

INTRODUCTION

Ommochrome pigments are a class of pigments that is widespread in insects and other arthropods. Their study has been mainly biochemical, and peaked in the '70s and '80s, just before the advent and rise of molecular biology (Linzen, 1974; Needham, 1974; Fuzeau-Braesch, 1972; Fuzeau-Braesch, 1985; Kayser, 1985). Scattered work has been carried out since then, related to the developmental biology of butterfly wing patterns (e.g. Koch, 1993; Nijhout, 1997; Reed and Nagy, 2005) or in the context of tryptophan metabolism (e.g. Han et al., 2007; Kato et al., 2006). The eye pigmentation pathway of *Drosophila* based on ommochrome synthesis and deposition has been extensively analyzed, mainly in the context of genetic and epigenetic work (e.g. Phillips and Forrest, 1980; Lloyd et al., 1998; Lloyd et al., 1999; Mackenzie et al., 2000). Ommochrome pigments are the main chromogenic class in the pathway from tryptophan. They range from gold (xanthommatin X) through red, purple and violet, up to black. The reduced form is red and the oxidised form usually yellow. The characteristic properties of ommochromes, i.e. redox behavior, absorption of ultraviolet and visible light, and low solubility, enable them not only to act as authentic functional pigments (eyes, integument), but also as an electron accepting or donating system and as metabolic end products (Needham, 1974). Ommochromes, principally xanthommatin, are widely distributed in arthropods as screening pigments in the accessory cells of the eyes and are also present in the retinula cells (Linzen, 1974).

The functions of ommochromes are diverse and several complementary and non-exclusive hypotheses have been suggested for their common occurrence; these have been well reviewed in the general references cited above. Hypothesis 1: the ommochrome pathway is the main pathway for avoiding excess accumulation of highly toxic tryptophan. Insects and other spiralian phyla have never possessed or have lost the simpler vertebrate catabolism pathway for tryptophan based on the glutarate pathway. Supporting this hypothesis is the observation that ommochrome formation is strongly correlated with the massive breakdown of proteins at the onset of metamorphosis. This is the oldest and most popular view for the existence of ommochromes. Hypothesis 2: it is believed that the major function of ommochrome eye pigments is protection of photosensitive visual cells against excessive scattered light, and also to protect them against photodestruction by intense ultraviolet light (Langer, 1975; Stavenga, 1989). Ommochromes participate in the antioxidative system in invertebrate photoreceptors, like melanin in the eyes of vertebrates (Dontsov et al., 1984; Dontsov, 1999; Ostrovsky et al., 1987; Sakina et al., 1987). The

ommochromes are effective inhibitors of free radical induced lipid peroxidation. Lipid peroxidation is also produced by photo-oxidation and is indicative of photoreceptor damage, manifested in the retina by deterioration of photoreceptor membranes (Ostrovsky and Fedorovich, 1994). Hypothesis 3: the color of ommochromes is believed to be used in signalling, mimicry and crypsis. This is the hypothesis supported by most of the community working on color changing insects such as stick insects and mantids (Fuzeau-Braesch, 1985), including *Mantis religiosa*, *Sphodromantis viridis* and *Locusta migratoria* (Vuillaume, 1968), and spiders (Rabaud, 1918; Rabaud, 1919; Gabritschevsky, 1927; Chittka, 2001; Schmalhofer, 2000; Théry and Casas, 2002; Heiling et al., 2003; Heiling et al., 2005; Théry et al., 2005; Théry, 2007).

The reversibly color changing crab-spiders of the family Thomisidae have been studied since 1891 (Heckel, 1891) with respect to pigmentation. Older works assumed that the yellow color of *Misumena vatia* was due to carotenoids (Millot, 1926), but ommochromes were later found to be the pigments responsible for this color change (Seligy, 1972). This spider is unusual, as it is able to change color reversibly, within a few days, from white to yellow and back. Both food and light quality have recently been found to increase the degree of color change, but the variability in the response level was very high, with many individuals remaining white despite strong stimuli (Théry, 2007). The matching to background that these spiders can produce is astonishing at times, for example below the discrimination ability of bees (Chittka, 2001; Théry and Casas, 2002; Théry et al., 2005). This form of mimetism has therefore been interpreted as potentially both a defensive (hiding from predators) and aggressive (luring prey) one. While the defensive color change hypothesis is still waiting for experimental and observational studies of predation by birds and may well eventually be proved wrong, bees and other flower-visiting insects are common prey. Finally, the occasional striking match between the colors of flowers and spider found in naturally occurring situations in the field is unlikely to be due to chance alone.

The aim of the present study was to describe the ultrastructural changes occurring during the color change from white to yellow, using several microscopic techniques, and to describe the ontogeny of the ommochrome pigment granules, in order to lay the necessary physiological foundations for the numerous statements about the adaptiveness of the color change that are being currently made.

MATERIALS AND METHODS

Adult female crab-spiders *Misumena vatia* (Araneae: Thomisidae) (Clerck 1757) were collected on flowers in the surroundings of Tours, France, during the spring and summer. Upon capture, they were maintained in clear plastic vials (7 cm high, 5 cm diameter) containing pieces of damp cotton and fed on houseflies (about one a week). We removed discarded prey items and cleaned the vials weekly.

For the morphological analysis, transmission electron and light microscopy were performed on the spiders following the technique described (Ribi, 1987). Briefly, a spider was fixed for 3 h in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH 7.3) with glucose and $CaCl_2$ added. Subsequently, the pieces were postfixed with buffered 1% osmium tetroxide for 1–2 h. After dehydration, they were embedded *via* propylene oxide in Durcupan ACM (Electron Microscopy Sciences no. 14040). Blocks were serially sectioned at 1.5–5 μm using glass knives mounted in a microtome. The sections were stained on a hot plate with Toluidine Blue–Basic Fuchsin or mounted unstained on a slide with DPX (Electron Microscopy Sciences no. 13510). The unstained sections were observed under a light microscope and a fluorescence microscope (using a USH102D burner, DM400 dichroic mirror, a BP330–385 excitation filter and a BA420 barrier filter; Olympus, Japan). The same sections were observed with a linear polarizer.

For electron microscopy, ultrathin sections were cut with an ultramicrotome using a diamond knife. The sections were doubly stained by uranyl acetate and lead citrate and observed using a JEOL 1010 transmission electron microscope.

RESULTS

General morphology

Adult females of *M. vatia* show a white (Fig. 1A) or yellow (Fig. 4) opaque opisthosoma, sometimes with a red stripe on the side. The prosoma is white or yellow translucent with opaque-white areas such as the eye contour, palps, chelicera and a small dorsal figure. A green stripe is also present at each side.

Below, we describe the sequence of tissues in the opisthosoma, starting from the cuticule and moving inward. First (and outermost), the cuticle is about 12–20 μm thick, with a folded external surface. A one-layered epithelium of epidermal cells (about 9–20 μm thick) with large irregular nuclei and a variety of granules in the cytoplasm is located below the cuticule. The adjacent epidermal cells are tightly bound to each other, without spaces between them. Beneath this epidermal layer are the hypodermic hemolymph sinus and a layer of muscles (Fig. 1C,D). Deeper is a thick layer of cells filled with brown granules (color observed in unstained preparations but fixed with osmium tetroxide). Ultrastructural studies revealed that these brown granules are crystals of guanine located in the peripheral intestinal diverticula (Fig. 1B,C,D).

The epidermal pigment granules

We first describe the content of epidermal cells for white spiders, then for yellow spiders and finally for the red stripes on both color forms. A synopsis of the different granule types is given in Table 1.

White spiders

In the opisthosoma, pigment granules are scattered in the cytoplasm of epidermal cells. They emit light-blue fluorescence and do not differentiate with the polarizer filter. They consist of poorly osmiophilic ellipsoidal granules, bound by a unit of membrane, with a section of 0.8 μm×1.4 μm (Fig. 2A,B). This type of pigment granule, denoted *type I*, is the only type of granule present in the epidermal cells in the white zone of the opisthosoma. Glycogen rosettes are scattered in whole cytoplasm (Fig. 2B, inset). We have not found Golgi bodies close to the granules. Striking structures of rough endoplasmic reticulum (RER), organized into several concentric rings, were found in close association with granules (Fig. 2C,D). The granules are enclosed by membranes, which are also intimately associated with those of the structure of RER (Fig. 2D, arrow). High densities of mitochondria are observed to be associated with these structures (Fig. 2C, arrows).

In the opaque white region of the prosoma, the cytoplasm of epidermal cells is rich in inclusions (Fig. 3A). They emit blue fluorescence (Fig. 3B) and are much brighter than the background when observed through a polarizer filter (Fig. 3C). Ultrastructural examination of the epidermal cell revealed that these are electron-dense granules with electron-lucent inclusions of micro-crystals

(Fig. 3D). Some granules are totally filled with microcrystals. Furthermore, cells located in the white translucent tegument of the prosoma contain *type I* granules only.

Yellow spiders

The nature of pigment granules in the epidermal cells of the opisthosoma of yellow spiders (Fig. 4) differs according to the intensity of the color. In light-yellow tinted spiders we found two types of granules: pale yellow granules and brown granules (Fig. 5A). We denote these granules as *type II* and *type III*, respectively. *Type II* granules emit light-blue fluorescence (Fig. 5B) and remain dark under observation with a polarizer filter. The fine structure of these granules revealed that they are not homogeneous, in contrast to the *type I* granules, and are confined within a unit of

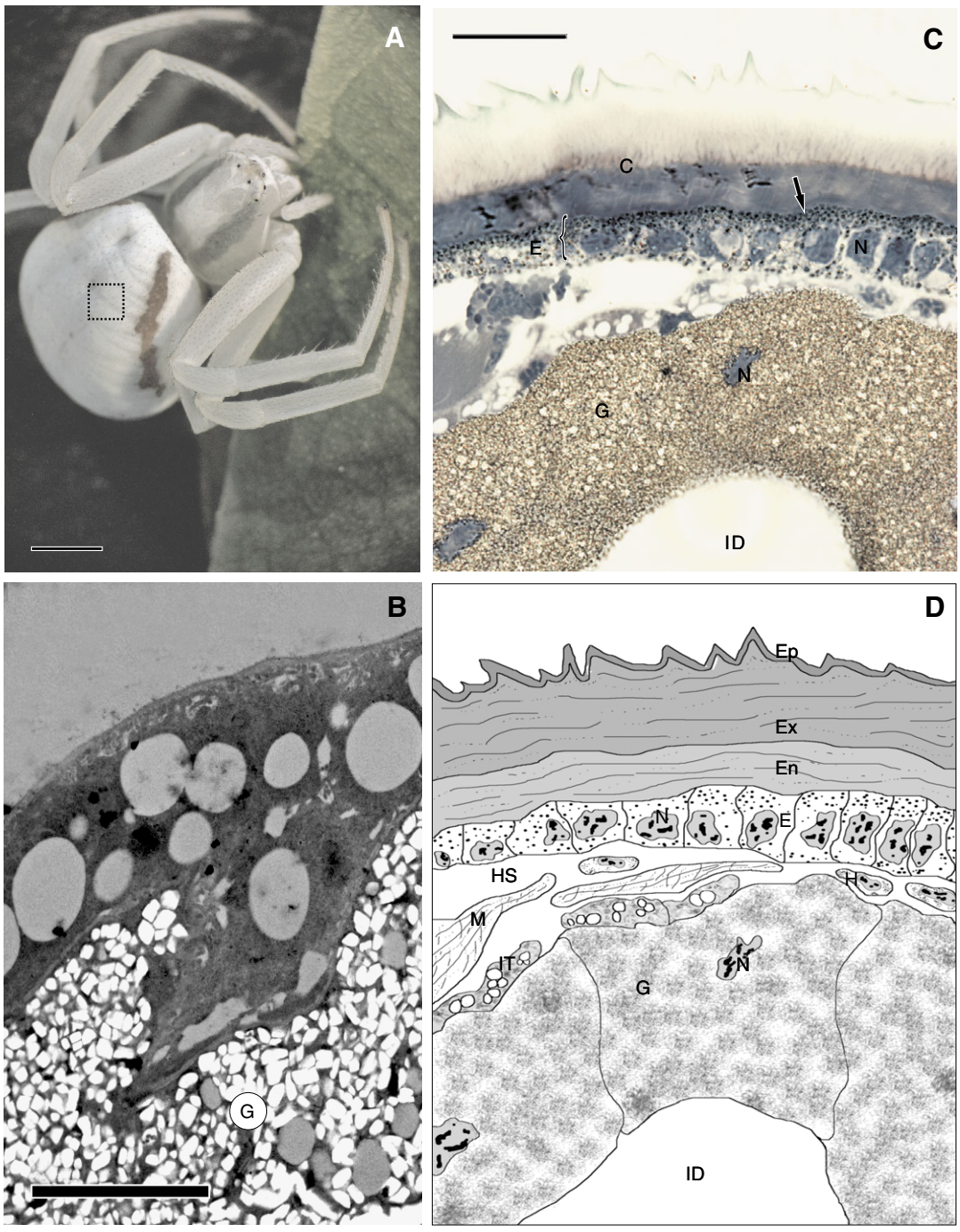

Fig. 1. (A) White *Misumena vatia* with red stripes (bar, 2 mm). (B) Electron micrograph of the peripheral guanocytes (G) with guanine crystals. Bar, 5 μm. (C) Light micrograph of stained section across the dorsal tegument of the opisthosoma (boxed in A) showing the cuticle (C) with the folded external surface, the epidermis (E) with granules (arrow), the guanocytes (G) and the intestinal diverticula (ID). Bar, 20 μm. (D) Schematic drawing of sections across the dorsal tegument of the opisthosoma, based upon the light microscopic observations. E, epidermis; En, endocuticle; Ep, epicuticle; Ex, exocuticle; H, hemocyte; HS, hypodermic hemolymph sinus, M, muscle; N, nucleus; IT, interstitial tissue, G, guanocyte; ID, intestinal diverticula.

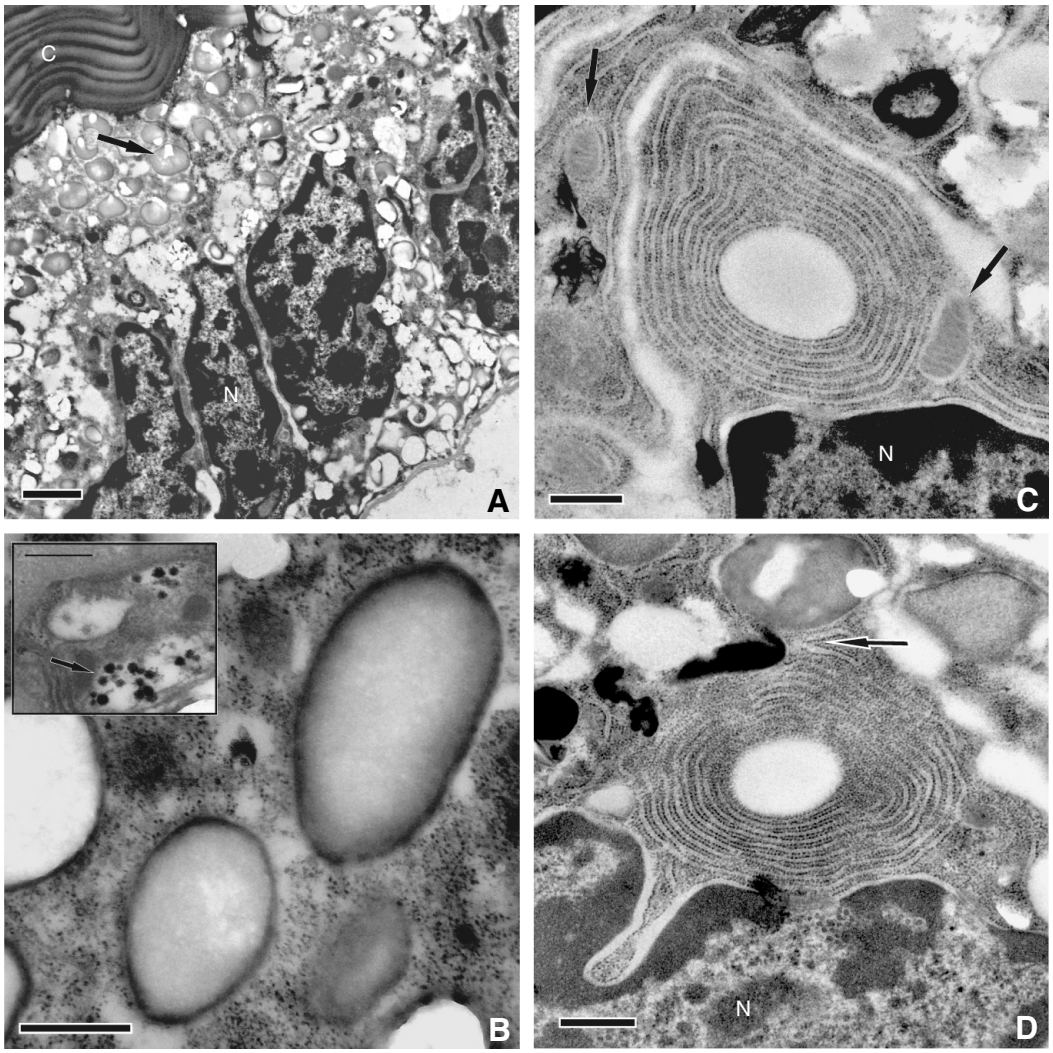

Fig. 2. Electron micrographs of the dorsal white tegument of the opisthosoma (boxed in Fig. 1A). (A) Epidermal cells with *type I* granules (arrow). Electron-lucent areas (holes) are present because granules are sometimes broken up by the microtome cutting. Bar, 2 μm. (B) Detail of *type I* granules (bar, 0.5 μm). The inset shows a detail of the abundant glycogen rosettes in the epidermal cell cytoplasm (arrow) (bar, 0.5 μm). (C) Detail of the structure of rough endoplasmic reticulum (RER) organized into several concentric rings. Note the presence of large number of mitochondria (arrows) related to these structures. Bar, 0.5 μm. (D) Another detail showing the RER structure in close association with granules (arrow). N, nucleus. Bar, 0.5 μm.

Table 1. Characteristics of the different types of epidermal pigment granules

Type of granules	Spider color	Color (OM)	Fluo.	Pol.	Morphology (EM)	Composition
Type I	White	Translucent	+	–	Ellipsoidal section, homogeneous, slightly osmiophilic content (0.8 μm×1.4 μm)	Progranule I (kynurenine?)
Type II	Light yellow	Pale yellow	+	–	Ellipsoidal to spherical section, heterogeneous, electron-opaque content with vesicular electro-lucent material (1–1.6 μm)	Progranule II (kynurenine + 3-OH-kynurenine, + ommochromes?)
Type III	Bright yellow, red stripe	Brown, red (stripe)	–	–	Spherical section, homogeneous, osmiophilic-electron-dense content (0.8–1 μm)	Ommochromes
Microcrystal inclusions	Prosoma (opaque-white region)	Pale brown	+	+	Spherical to irregular section, electrodense content with clear polygonal inclusions (0.8–1 μm)	Uric acid

OM, optical microscopy; EM, electron microscopy; Fluo., fluorescent material when viewed under UV light; pol., birefringence property measured using a light polarizer.

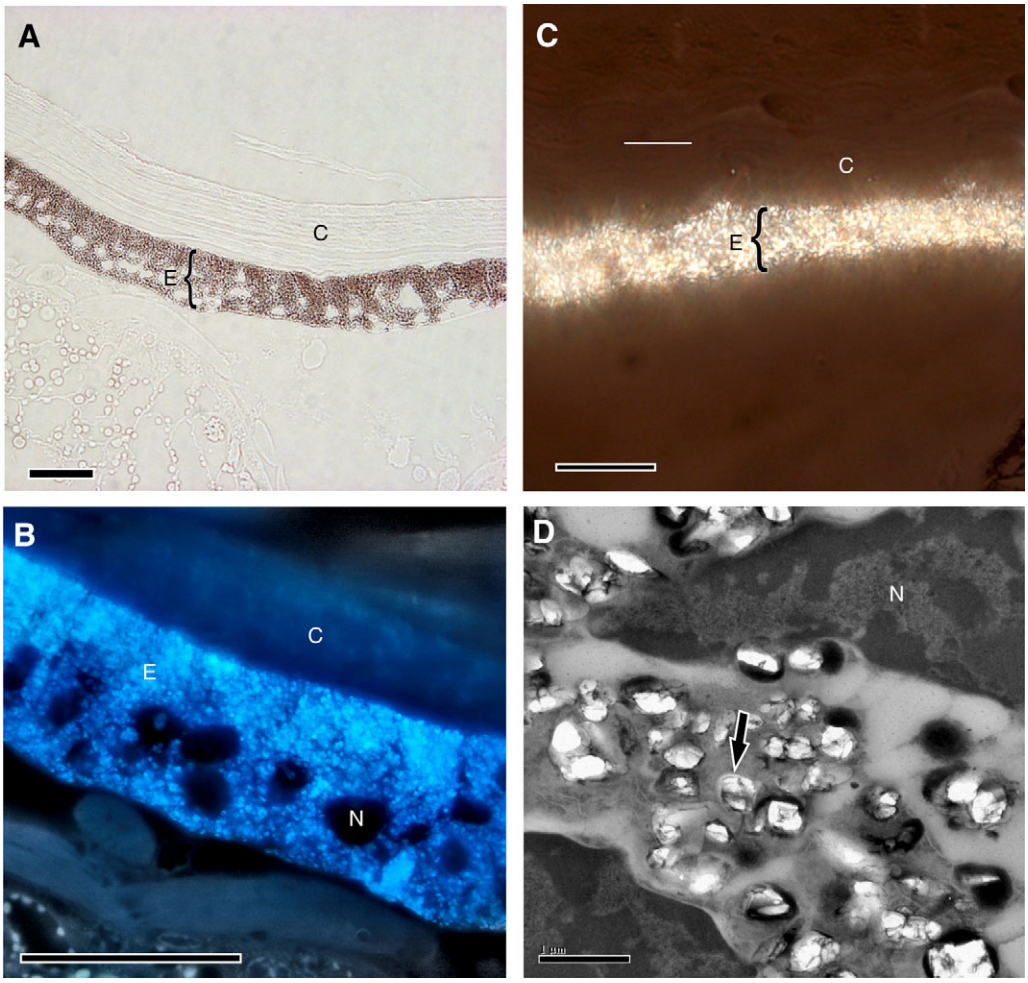

Fig. 3. Sections of the opaque-white region of the prosoma of a white spider. (A) Unstained section showing the granules filling the epidermal cells (bar, 30 μm). (B) Same region of tegument as in A, observed under UV light. The granules are strongly autofluorescent (bar, 10 μm). (C) Same region as A and B, observed through a linear polarizer. Numerous granules appear as brilliant (birefringent) against the dark background (bar, 20 μm). (D) Electron micrograph of the epidermal cells of the same region, showing the granules full of microcrystal inclusions (arrow) (bar, 1 μm). C, cuticle; E, epidermis; N, nucleus.

membrane (Fig. 5C). They revealed an heterogeneous electron-opaque content with vesicular electro-lucent material. We observed that some granules contained small vesicles only, whereas others contained some small vesicles surrounding a bigger one located in the centre of the granule. Other granules have an electron-lucent center and an electron-dense ring border (Fig. 5C). A high density of glycogen rosettes occurs in the cytoplasm of the cell (Fig. 5F). Golgi bodies and smooth endoplasmic reticulum are frequently present (Fig. 5C inset, F). Brown granules of *type III* are the only ones present in bright yellow spiders (Fig. 5D). They can be found in the same cell of yellowing spiders as *type II*. They emit no fluorescence and remain unaffected by the polarizer filter. The *type III* granules are electron-dense, with a diameter of 0.8–1 μm and a spherical section. Their content is homogeneous and they are enclosed by membranes (Fig. 5E,F). They are sometimes broken up by the microtome cutting.

Fig. 4. Yellow *M. vatia*.

Epidermal cells of the prosoma of yellow spiders do differ in their pigment content according to the location of the cell. Cells in the opaque region contain two types of granules: *type III* granules, located at the top of the cells, and granules with microcrystal inclusions (already described in the prosoma of the white spider), located at the base of the cell (Fig. 6A–D). Sometimes microcrystals tend to group together, forming structures surrounded by rough endoplasmic reticulum (Fig. 6E). Cells located in the yellow translucent tegument of the prosoma contain *type III* granules only.

Red stripes in white and yellow spiders

The epidermal cells are rich in granules in the red stripes. In the white spider, two types of granules were observed: dark red granules in the basal and medial zone of the cell, and translucent granules in the apical zone of the cell (Fig. 7A). Observations of this region by fluorescence microscopy revealed that the apical and medial zones of the cell emit light-blue fluorescence (*type I* granules), whereas the basal zone emits no fluorescence (*type III* granules) (Fig. 7B). The ultrastructure of this region confirmed the presence of two types of granules: *type III* at the basal region of the cell and *type I* at the apical region (Fig. 7C).

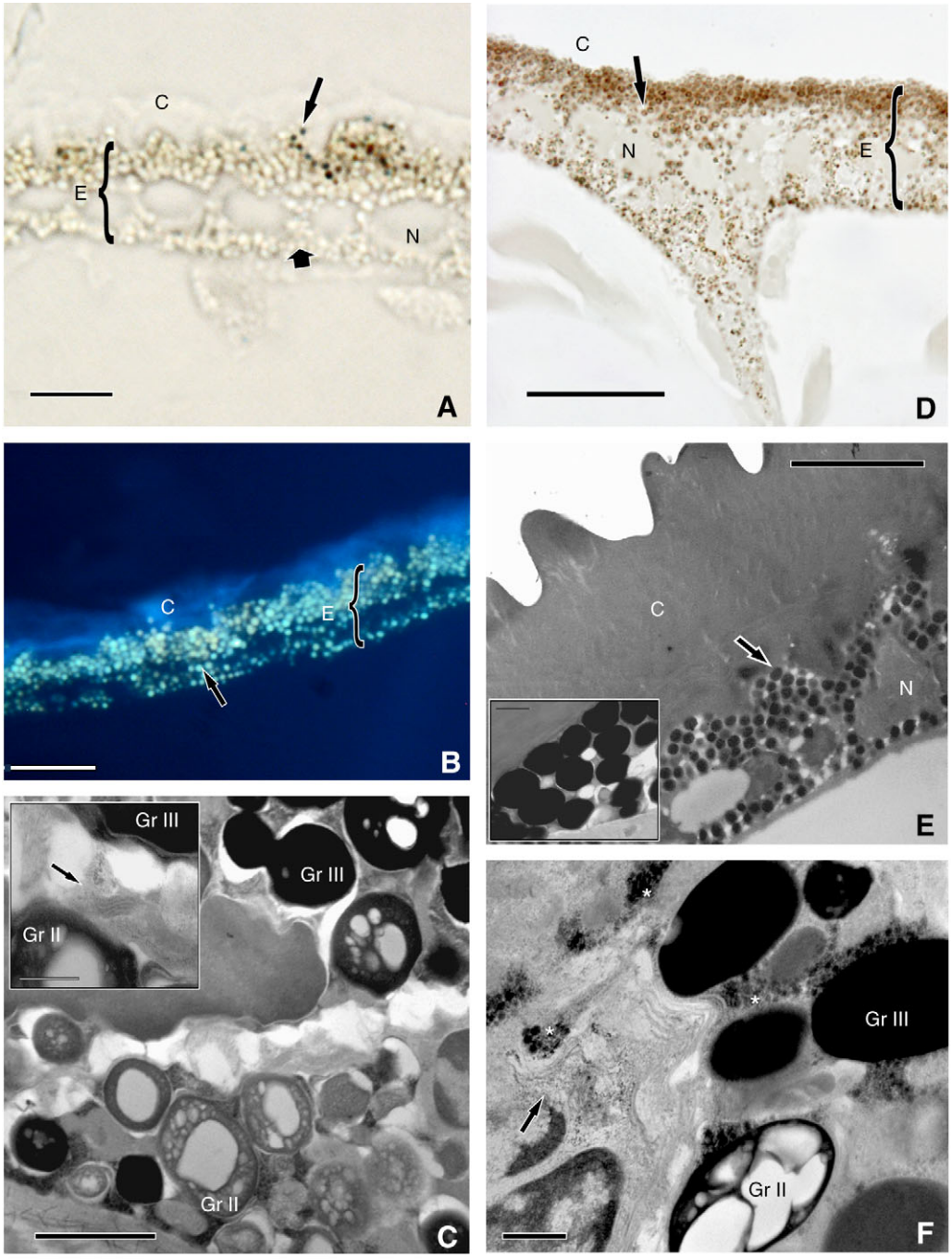

Fig. 5. Micrographs of cross sections of the tegument of the yellow spider. (A) Unstained section of the light-yellow spider, showing the presence of two types of granules: *type II* (short, thick arrow) and *type III* (long, thin arrow) (bar, 10 μm). (B) Same region as in A observed under UV light. The arrow indicates the fluorescent *type II* granules. Note the absence of fluorescence stemming from the isolated *type III* granules (bar, 10 μm). (C) Electron micrograph of the same region as A and B showing in detail the granules *type II* and *type III* (ommochromes) (bar, 2 μm). The inset shows a detail of a Golgi body (arrow) (bar, 0.5 μm). (D) Unstained section of the bright-yellow spider, with the epidermal cells full of *type III* granules (arrow) (bar, 20 μm). (E) Electron micrograph of the same region as D. Note the high concentration of the homogeneous granules *type III* (arrow) filling the cell (bar, 10 μm). The inset shows a detail of the *type III* granules (bar, 1 μm). (F) Detail of the epidermal cell cytoplasm showing the abundance of glycogen rosettes (asterisks) and a Golgi region (arrow) (bar, 0.5 μm). C, cuticle; E, epidermal cell layer; N, nucleus; Gr II, granule *type II*; Gr III, granule *type III*.

In the red stripe of the yellow spiders, yellow and red tinted granules are mixed through the whole cell (Fig. 7D). This zone emits no fluorescence (both types of granules are *type III* granules) (Fig. 7E, left). When observed through a linear polarizer, the granules did not behave differently from the surroundings. These characteristics are typical of *type III*. The ultrastructural study of this region confirmed the presence of *type III* granules only (Fig. 7F, left).

DISCUSSION

White coloration produced by guanine and uric acid inclusions

The colorless cuticle of the opisthosoma transmits the white color of guanine crystals located beneath the epidermis (Millot, 1926; Weigel, 1941; Seitz, 1972; Oxford, 1998). Guanine, a purine compound stored in intestinal cells, was the only pigment so far identified as responsible for the white coloration of spiders (Holl, 1987; Oxford and Gillespie, 1998). Because guanine emits no fluorescence (under our lightning conditions) and is not birefringent, we rule out its presence in the prosoma region, in which we observed a second white pigment, different from guanine. Pterin, a very common pigment in the white tegument of insects, is not present in detectable quantities in the epidermis of spiders, particularly *M. vatia* (Seligy, 1972). The granules that we observed were electron-dense, with electron-lucent inclusions of microcrystals with a characteristic fluorescence and birefringence. This suggests that these abundant granules contain uric acid. Guanine and uric acid are still present in yellow spiders. The combination of a yellow granule layer (*type III*) with crystals of either nature leads to the brilliant yellow color we observe.

Chemical identity of the pigment granules: ommochromes and their precursors

We identified two types of progranules (*type I* and *type II*) and a complete granule (*type III*), and their developmental relationship (Fig. 8). These granule types can occur seperately or combined in the same cell. The characteristics and structure of the *type III* granules allow us to conclude that they are carriers of ommochrome pigment, which have been extensively chemically characterized (Stamm Menendez and Galarza Basanta, 1961; Linzen, 1967; Linzen, 1974; Seligy, 1972; Holl, 1987). Ommochromes are derivatives of the amino acid trytophan *via*

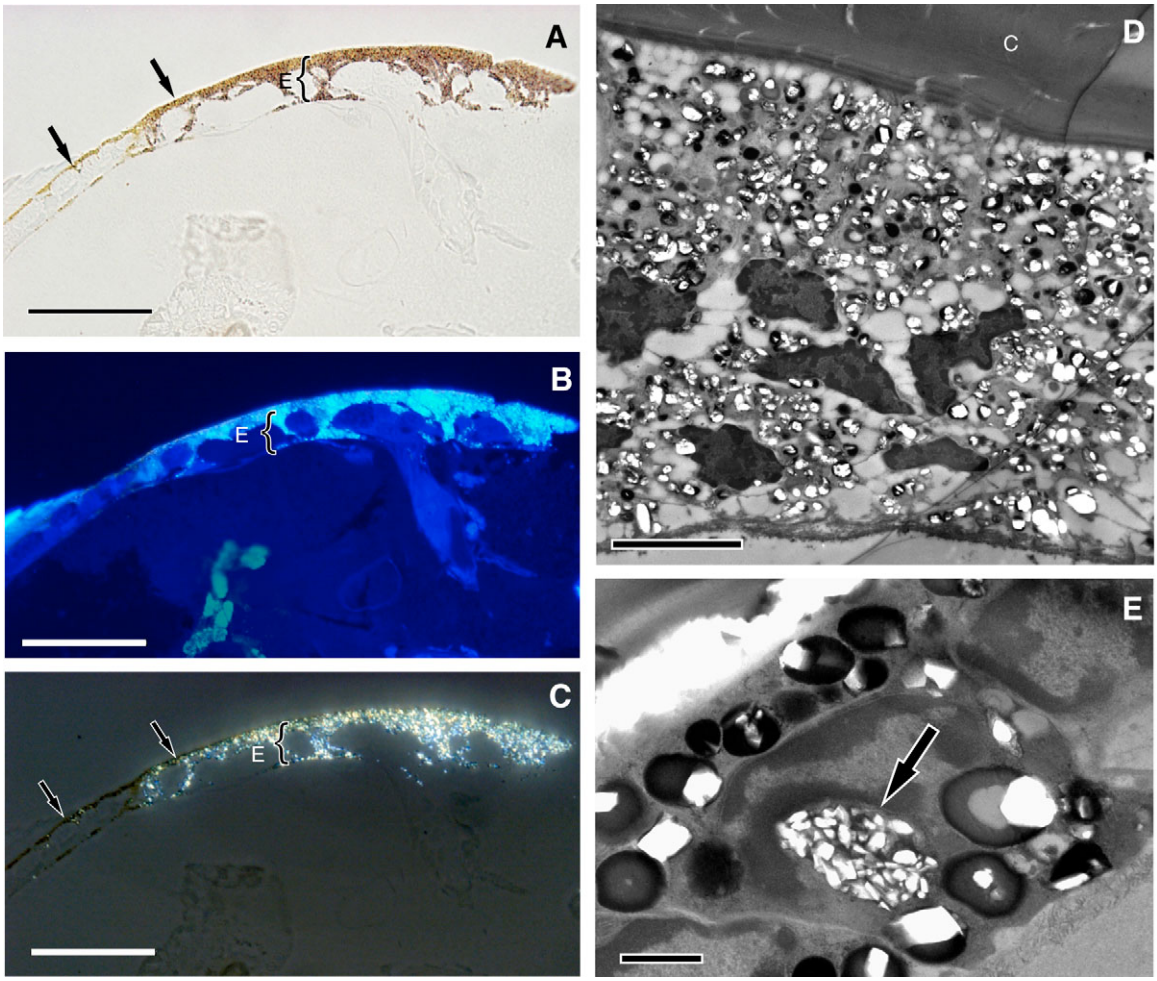

Fig. 6. Sections of the opaque-white region of the prosoma of the yellow spider. (A) Unstained section showing the distribution of the yellow *type III* granules (arrow) over the granules with microcrystal inclusions (bar, 50 μm). (B) Same region of tegument as in A observed under UV light, showing the autoflorescence of the granules with microcrystal inclusions (bar, 50 μm). (C) Same region as A and B, observed through a linear polarizer. The granules with microcrystal inclusions were birefringent whereas the *type III* granules (ommochromes) remained dark (arrows) (bar, 50 μm). (D) Electron micrograph of the epidermal cells of the same region, showing the granules full of inclusions of microcrystals (bar, 5 μm). (E) Detail of D showing a structure of accumulation of microcrystals (arrow) (bar, 1 μm). C, cuticle; E, epidermis.

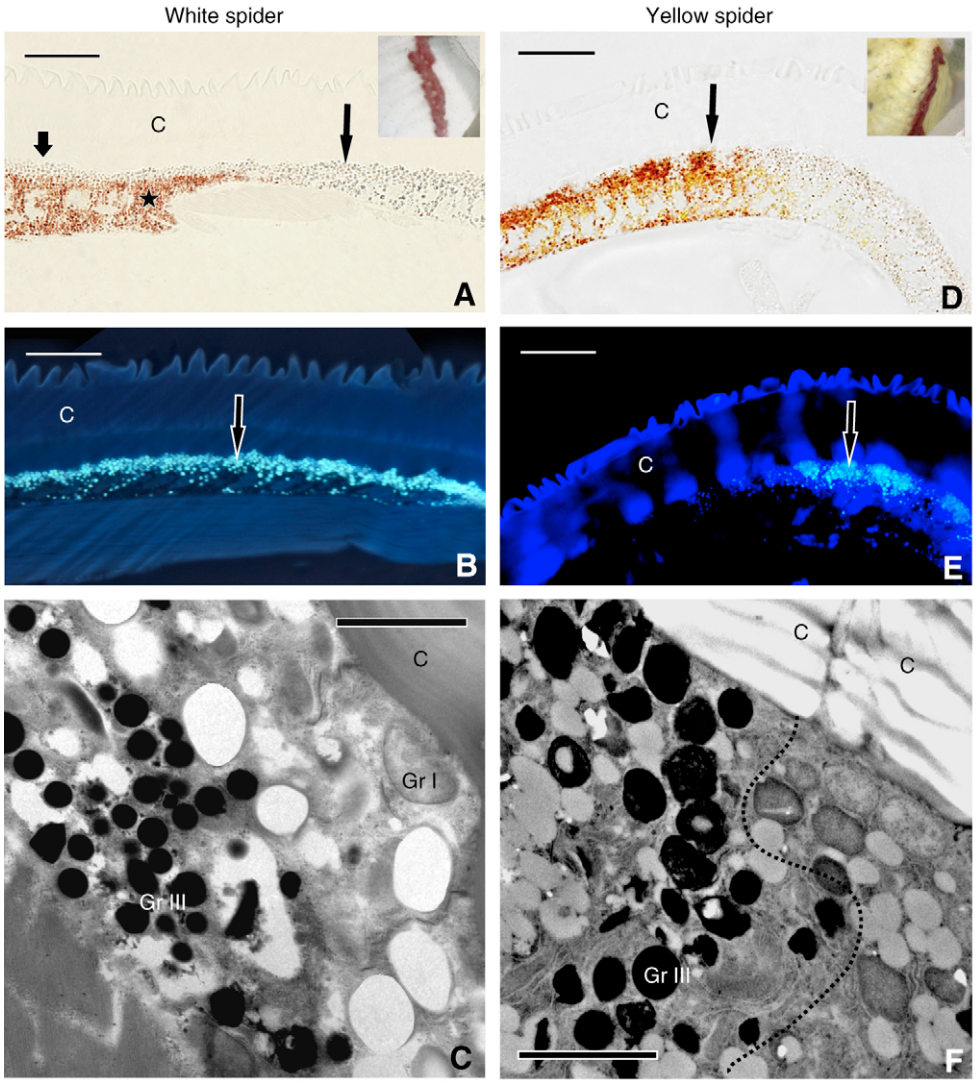

Fig. 7. Micrographs of cross sections of the red stripe zone. (A–C) White spider. (A) Unstained section. The red zone (left) and the white zone (right) of the epidermis. Two types of granules in the red zone are shown: the basal red *type III* (ommochromes) (asterisk) and *type I* granules (short, thick arrow) in the apical region of the cell. Only *type I* granules (long, thin arrow) are present in the white zone (bar, 20 μm). (B) Section of the red stripe zone showing fluorescent *type I* granules (arrow) in the apical and medial region of the epidermis. Note the absence of fluorescence from nuclei and the *type III* granules (basal region of the epidermis) (bar, 20 μm). (C) Electron micrograph of the red stripe showing a detail of the distribution of the *type I* and *type III* granules (bar, 2 μm). (D,E,F) Yellow spider. (D) The red zone (left; arrow) and the yellow zone (right) of the epidermis. Only *type III* granules (red and yellow) were observed in the red zone (bar, 20 μm). (E) Same region as in D, observed under UV light. Only the *type II* granules of the yellow zone show autofluorescence (arrow, half right of the picture) (bar, 20 μm). (F) Electron micrograph showing a detail of the same region as D and E. The dotted line delimits the red stripe and yellow regions (bar, 2 μm). C, cuticle; Gr I, granule *type I*; Gr III, granule *type III*.

kynurenine and 3-OH-kynurenine. They are responsible for yellow (oxidized xanthommatin) and red (reduced form) colors found in many invertebrates (Needham, 1974; Oxford and Gillespie, 1998). The different granules that we found in the epidermis of the spider are very similar to the ommochrome granules type 1–3 described in locust epidermis (Bouthier and Lhonoré, 1984). Five morphological categories were defined, corresponding to different developmental stages of pigment granules. Ommochromes are progressively deposited onto the homogeneous matrix of type 1 progranules of unknown chemical nature. The final granule (type 3) contains the true pigment. Granules types 4 and 5 corresponded to successive steps of mineral deposition in the matrix (calcium phosphate and uric microcrystals) (Bouthier and Lhonoré, 1984).

Chemical identification of the final granule type, ommochromes, enabled us to work back through their metabolic pathway to hypothesize the chemical composition of the progranules. All metabolites of the ommochrome pathway can easily be detected under ultraviolet light, as they retain the fluorescence due to their aromatic ring (Linzen, 1967). The natural fluorescence of kynurenine and 3-OH-kynurenine, the two major precursors of ommochromes, provides a convenient means of localizing these metabolites intracellularly. The characteristic fluorescence and the fine structure of progranules of *type I* and *type II* suggest that they could contain both precursors. The content of *type I* progranules is homogeneous, so we assume that *type I* progranules contain kynurenine only. During the change in color from white to yellow, the vesiculated progranule *type II* could be an intermediate form between the

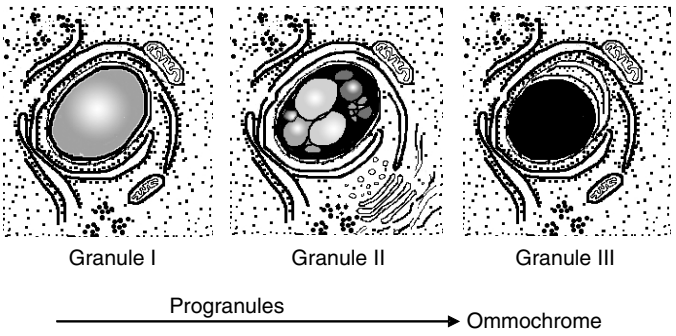

Fig. 8. Development of progranules (*types I and II*) into pigment granules of ommochromes (*type III*). Ommochromes are progressively deposited onto a homogeneous matrix of *type I* progranules (white spider), through an intermediate state of vesiculated progranules *type II* (light yellow spider). The progranules then decrease slightly in size and form the ommochrome pigment granules (*type III*) (bright yellow spider).

progranule *type I* and the ommochrome granules. The different states of the *type II* progranules (heterogeneous content) suggest that the vesicles could contain both kynurenine and 3-OH-kynurenine, and also the final product (ommochrome) in their electro-dense regions.

There is ample evidence from other studies to support our inference regarding the chemical identity of the progranules. In the stick insect *Carausius morosus*, for example, all the metabolites of the ommochrome pathway are found in the epidermis (Stratakis, 1980). All tryptophan metabolites have been shown to be present in the eyes of *Apis mellifera* (Dustmann, 1975). In the insects *Schistocerca gregaria* and *C. morosus*, 3-OH-kynurenine was found to occur in the epidermis (Pinamonti et al., 1973; Stratakis, 1980). Finally, the spiders *Argiope aurantia* and *A. trifasciata* accumulate both kynurenine and 3-OH-kynurenine in their opisthosomal hypodermis (Seligy, 1972). Our current biochemical HPLC studies tend to confirm the presence of large amounts of these two metabolites in *M. vatia* epidermis (J.C., unpublished observation). Even though our hypothesis is supported by morphological observations, further work is necessary to elucidate the biochemical nature of the granules.

The granule formation is associated to endoplasmic reticulum

The cytological origin of ommochrome pigment granules has often been associated with Golgi vesicles. In particular, Shoup (Shoup, 1966) reported the presence of immature granules adjacent to Golgi regions of developing fly eyes and concluded that these granules originate as vesicular secretions of Golgi apparatus. By contrast, Fudge (Fudge, 1967) observed that the granules arise from the small cisternae of the smooth endoplasmic reticulum (SER) in the eyes of *Drosophila melanogaster*. Taking an intermediate position, Bouthier and Lhonoré (Bouthier and Lhonoré, 1984) related the formation of initial progranules with Golgi vesicles found in the epidermal cells of *Locusta migratoria cinerascens*, but could not conclude whether these progranules were derived from dictyosomes or from rough endoplasmic reticulum (RER). Recently, a unique pathway for screening granule formation in the retina of the opilion *Eumesosoma roeweri* was proposed (Johnson and Gordon, 1990). An endoplasmic reticulum network is at work in the formation of each granule. Each site is composed of concentric, interconnected rings of SER that are filled with spherical pigment particles. The formation of screening pigment granules occurs in the middle of these rings and begins with the release of particles from the innermost rings of carrier reticulum.

A common origin of vertebrate pigment cells, melanophores, xanthophores and iridophores, was proposed by Bagnara et al. (Bagnara et al., 1979). These cells contain pigmentary organelles known, respectively, as melanosomes (melanins), pterinosomes (pteridines) and reflecting platelets (purines). These authors suggest the existence of a primordial organelle derived from the endoplasmic reticulum. This preorganelle may be a vesicle formed from the RER, and may represent an early structural component in the genesis of each pigmentary organelle. In the formation of melanosomes, the premelanosome is derived from cisternae of the RER, and then fuses with vesicles containing tyrosinase enzymes, considered to be derived ultimately from the Golgi complex (see also Palumbo et al., 1997).

The ontogeny of ommochrome granules bears strong similarities to that described above for vertebrate pigment cells. We observed a strong relationship between the structure of concentric rings of RER, the relative high density of mitochondria and the glycogen rosettes with the pigment granules in *M. vatia*. The external layer of the ring structure appears to be continuous with the outer membrane of a granule. While there is a close morphological analogy between the RER structure of *M. vatia* and the SER structure observed by Johnson and Gordon (Johnson and Gordon, 1990), the presence of RER suggests a closer functional analogy with the model described by Bagnara et al. (Bagnara et al., 1979). The *type I* granules present in the white spider are probably primordial vesicles derived from RER. We have so far failed to detect Golgi vesicles, despite intensive search in the vicinity of progranules of *type I*. However, we found that Golgi bodies are frequently present near the *type II* granules (yellowing spiders), which suggests that they might have a role in the transformation of progranules to ommochrome pigment granules.

Mechanisms and significance of color change

Our understanding of pigment granule development and the presence of different stages of granule formation in different color morphs enable us to revisit the three main hypotheses for the arthropod ommochrome formation described in the Introduction.

The epidermis of white spiders is full of granules containing ommochrome precursors, most likely kynurenine. White spiders with red stripes have large amounts of ommochromes localised precisely and only in these stripes. Hence, the absence of a change of color from white to yellow is not due to a lack of precursors, nor a lack of enzymes [as found in the white eyes clones of *Drosophila melanogaster* (Mackenzie et al., 2000)]. This clear conclusion invalidates the common hypothesis stating that the ommochrome production is due to the necessity of avoiding high cellular concentrations of tryptophan (hypothesis 1), since it is already neutralized as the ommochrome precursor in granules of *type I* (before changing to yellow). Storing this toxic compound as kynurenine might be sufficient. However, hypothesis 1 could hold true for other tissues or organs, such as Malpighian tubules. The photoprotection role of ommochromes, another common hypothesis for the role of ommochromes due to their widespread occurrence as screening pigments in insect eyes (hypothesis 2), deserves much more attention. Indeed, *M. vatia* is quite original in being both exposed for days to direct solar radiation on the top of flowers and in having a transparent cuticle exposing the epidermal cells to direct radiation. Ommochrome precursors could however be sufficient as screening pigments, as in the group of *chartreuse* mutants of *Apis mellifica* (Linzen, 1974). Indeed, the mutant group accumulates the yellow tinted but still translucent 3-OH-kynurenine in a granular form in the pigment cells of the compound

eyes. That pigment precursor therefore assumes a pigment function (Linzen, 1974). The intensity of the yellow hue of spiders, a result of the mix between 3-OH-kynurenine and ommochromes, might reflect the amount of screening against radiation. As indicated in the Introduction, in addition to this optical function, given their antioxidant properties, ommochromes constitute protective agents against UV-induced photodamage.

The final and most favored hypothesis from the ecologist's point of view for the formation of ommochromes (hypothesis 3) is mimetism and crypsis. A cost–benefit analysis of ommochrome production is, however, required to understand the fitness gain from the change of color in an evolutionary context. It can only be based on a precise nutritional budget, at present lacking for this class of pigment. It also requires the measurement of some fitness-related trait, such as increased fecundity, survival or simply higher prey capture rate, as a function of the degree of flower color matching, a main piece still missing in the puzzle. Furthermore, while the basic metabolic pathway and enzymes for the anabolism of ommochromes are partially identified, the catabolism of these pigment granules, which is relevant when spiders revert from yellow to white, is unknown. We therefore lack a dynamic vision of this highly reversible phenomenon. In conclusion, any claim concerning physiological costs and ecological benefits of color change must be considered with extreme care. While our work tends to reject one hypothesis and support another, too many key assumptions remain untested for its acceptance and decisions about further hypotheses. Results from ultrastructural studies offer us a sobering reminder of how tenuous the functional basis is of most of the discussions and claims over the last century.

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