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DEVELOPMENTAL CHANGES IN THE INTERFERENCE REFLECTORS AND COLORATIONS OF TIGER BEETLES (*CICINDELA*)

BY T. D. SCHULTZ AND M. A. RANKIN

Department of Zoology, University of Texas, Austin, Texas 78712, U.S.A.

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SUMMARY

Samples of cicindelid cuticle were examined at various stages of adult ecdysis. The multilayered potential reflector was secreted in the initial stages of the moult, verifying that it is not tectocuticle and supporting the contention that it is a form of inner epicuticle. At early stages of ecdysis, the electron-dense layers were visible only when the section was post-stained. During post-ecdysial colour development, the dense layer increased in inherent electron density. Concurrently, the reflector increased in refractive index and the interference coloration increased in intensity and wavelength of maximum reflectance. Black pigment was also deposited simultaneously within the outer portion of the cuticle. It is proposed that electron-dense material was deposited *in situ* within the inner epicuticle after ecdysis, thereby increasing the wavelength and reflectance of interference colour.

INTRODUCTION

Schulze (1913) and Stegemann (1930) described the iridescent colour-producing layer of tiger beetle (Cicindelidae) cuticle as a 'Sekretschicht' or secretory layer produced by epidermal glands after ecdysis. This layer appeared as a superficial, thin, dark layer in cross-sections of elytra under optical microscopy (Stegemann, 1930). A gradual increase in thickness of this dark layer was interpreted as the discharge of a pigmented fluid over the surface of the pharate exoskeleton. Subsequently, the 'Sekretschicht' has been assumed to be a form of tectocuticle (Richards, 1951) or cement layer (Wigglesworth, 1972).

Detailed observations of post-ecdysial colour changes in cicindelids were made by Shelford (1917) and Willis (1967). In all of the species studied, the interference colours of the cuticle progressed from short to long wavelength colours during a 48-h period following emergence from the pupal exuvia. Simultaneously, the cuticle became pigmented and opaque to transmitted light. Histological or ultrastructural changes were not considered in these two studies.

Key words: Interference colour, epicuticle, *Cicindela*.

Schultz & Rankin (1985) identified the source of structural colours in tiger beetle (*Cicindela*) integument as epicuticular by virtue of its location, ultrastructure and reaction to solvents. The inner epicuticle consists of alternating layers of electron-dense and electron-lucent material which serve as a multilayer interference reflector. The reflective cuticle is located in the outer 2 μm of the exoskeleton. Cuticular layers below the reflective cuticle exhibit exocuticular characteristics.

In this study, the development of the cuticular reflector was investigated. Since the reflected colour directly relates to the optical thickness of the reflecting layers, development of these layers may be observed through the development of the interference coloration. The ultrastructure of developing cuticle was examined at different stages of colour development by transmission electron microscopy. The time of deposition of the reflecting layers also served to identify the layer according to current concepts of cuticle development and structure.

MATERIALS AND METHODS

Populations of larval and adult *C. scutellaris* Vaurie and *C. splendida* Hentz were counted over a period of 2 years (1979–1980) in Bastrop State Park, Texas. The approximate date of pupation was determined by census data and observations of larval activity.

Third instar larvae and pupae were excavated from their burrows and reared to adulthood in the laboratory. Larvae were placed in glass tubes, 70 cm × 30 mm. The extreme length was necessary for *C. scutellaris* whose burrows often exceed 50 cm. Tubes were packed with specific soils from oviposition sites in the field (Patilo soils for *C. scutellaris*, Axtell soils for *C. splendida*), and were placed vertically in a growth chamber with a photoperiod of 12·5 h light and 11·5 h dark. The sand was moistened every 6 days from the top of the tube. Larvae were fed *Tenebrio* larvae and adult *Tribolium confusum*.

Pupae were placed in opaque plastic cups. Each cup was padded with cotton, which formed an artificial pupal chamber. The artificial chamber was critical in ensuring that the imago would shed the pupal exuvia successfully. The cotton was moistened periodically with a solution of 1·5 % H_2O_2 to prevent mould developing. The development of adult colour was observed under a dissecting microscope.

Elytral cuticle was cut from developing *C. scutellaris* 4 days before ecdysis (10 days after pupation), 12 h after ecdysis, and 14 days after the moult. Strips of cuticle were prepared for transmission electron microscopy (TEM) as described previously (Schultz & Rankin, 1985). Sections of pharate and adult cuticle were both post-stained and left unstained. Post-staining consisted of 5 min in 1 % uranyl acetate in 50 % ethanol, followed by 5 min in lead citrate. All samples were examined under a Zeiss 10CA transmission electron microscope. Thin sections were also observed under optical microscopy.

RESULTS

The colour development of the tiger beetles, *C. splendida* and *C. scutellaris*, showed a progressive increase in peak reflected wavelength to the mature adult colour. Since the reflector is composed of multiple layers (Schultz & Rankin, 1985), there

must be either a gradual addition of layers which reflect longer wavelengths, or a gradual increase in the optimal thickness of the layers themselves. Electron microscopy, coordinated with observations of colour development, supported the latter interpretation. Since the ultimate colour of *C. splendida* is red, the sequence of colour development was longer, but essentially the same as that of *C. scutellaris*. For the sake of brevity, only the observations of *C. scutellaris* will be described.

The pharate elytron, 4 days prior to ecdysis, was pale white or cream with a slight violet iridescence. Electron micrographs revealed that the presumptive epicuticle (region *A*), and part of the exocuticle (region *B*), had already been deposited by this time (Fig. 1). The epicuticle was already composed of a full complement of bands, but the presumptive dense bands were thinner (15–40 nm thick) than they appear in mature cuticle, and were revealed only by post-staining (see Methods). In unstained sections, light 'negative shadows' appeared within the boundaries of the dark bands (layer *D* in Fig. 1). These could be distinguished from the grainy appearance of the potential electron-lucent bands (layer *L*). With post-staining, however, these areas appeared very dense, black and finely grained (Fig. 2). An electron-dense strip, 30 nm thick, was present in both stained and unstained sections at the outer border of the cuticle. The entire thickness of the epicuticle at this stage was 50–60 % thinner than in mature cuticle. No evidence of pigmentation was observable in optical cross-sections of the pharate elytron.

By the completion of ecdysis, the elytral colour had not changed substantially. Over the next 8 h, the basic colour of the cuticle darkened from white to a golden straw. The elytra were not yet pigmented, but displayed a distinctive violet hue. From 8 to 12 h after ecdysis, the iridescence became predominantly blue, and spread spatially over the elytron from base to apex. Simultaneously, the elytron blackened and became opaque. Pigmentation progressed through the elytron from base to apex, enhancing the lustre of the iridescence. By the 12th hour, all but the apical quarter of the elytron was pigmented, and it reflected a rich, blue colour. The apex was still violet and somewhat transparent.

Micrographs of cuticle 12 h after ecdysis showed that the epicuticle had assumed its surface microsculpture (Schultz & Rankin, 1985), and the outer exocuticle was fully formed (layers *A* and *B* in Fig. 3). Underlying layers of exocuticle (layer *C* in Fig. 3) were evident, but the formation of 'plywood' mesocuticle was yet to be initiated. The limits of the dense epicuticular bands were defined more clearly and expanded to approximately 80 % of their eventual thickness. However, they lacked the very dark granularity apparent in mature cuticle. Electron density decreased from outer to inner layers.

The elytra were entirely blue and pigmented 24 h after shedding the pupal exuvium. Over the following 6 h to 5 days the iridescent hue became progressively more green. All areas of the beetle became fully pigmented. The resistance of the elytron to superficial tears increased during this period, but the elytron did not become rigid and hardened for several days. In the field, this occurs after the adult has emerged from its pupal chamber. Drying undoubtedly facilitates elytral hardening. By the 14th day, the elytra and colour were developed fully.

Electron micrographs of mature cuticle show fully formed epicuticle, outer exocuticle and procuticular layers (Fig. 4). Although post-staining enhances the resolution

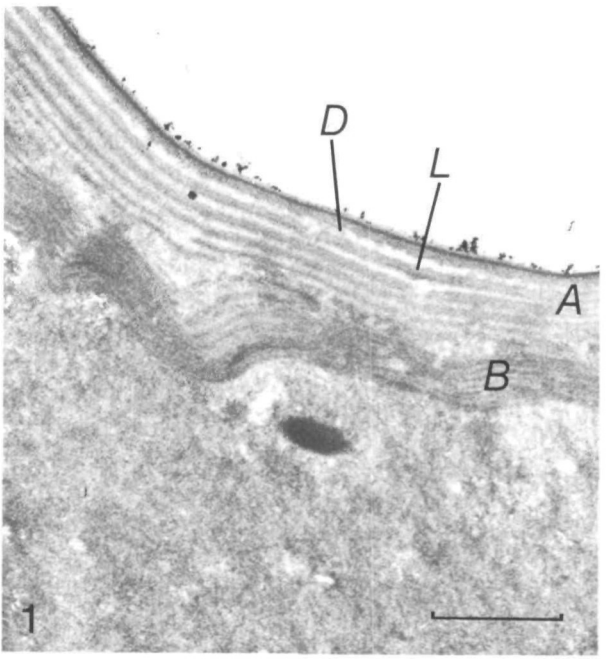

Fig. 1. Transmission electron micrograph of cross-section of pharate elytral cuticle 10 days after pupation. This section was not post-stained. The epicuticle (*A*) has been deposited and exhibits a multilayered ultrastructure. If not post-stained, the potential dense layers (*D*) appear less dense than the potential *L* layers. The outer exocuticle (*B*) has been partially deposited. Scale bar, 1 μm.

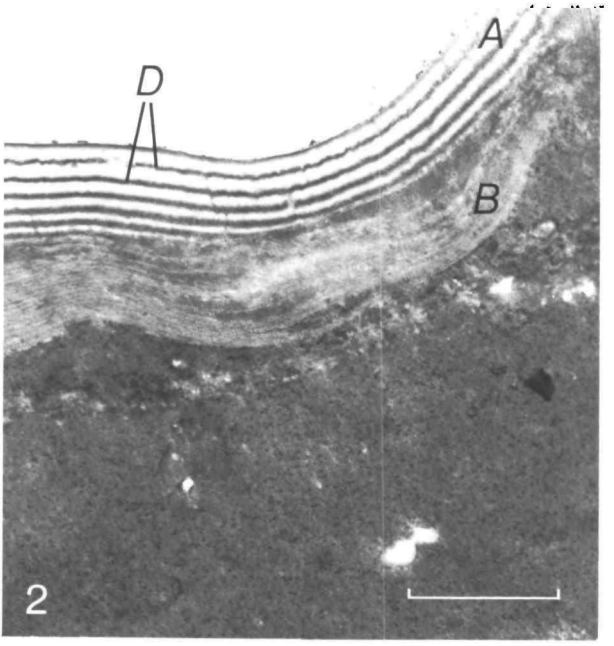

Fig. 2. Transmission electron micrograph of cross-section of pharate elytral cuticle 10 days after pupation. This section was taken from the same tissue as was the section in Fig. 1, but was post-stained with lead citrate and uranyl acetate. Structural features are the same, except that the potential *D* layers appear very dense and granulate. *A*, epicuticle; *B*, outer exocuticle. Scale bar, 1 μm.

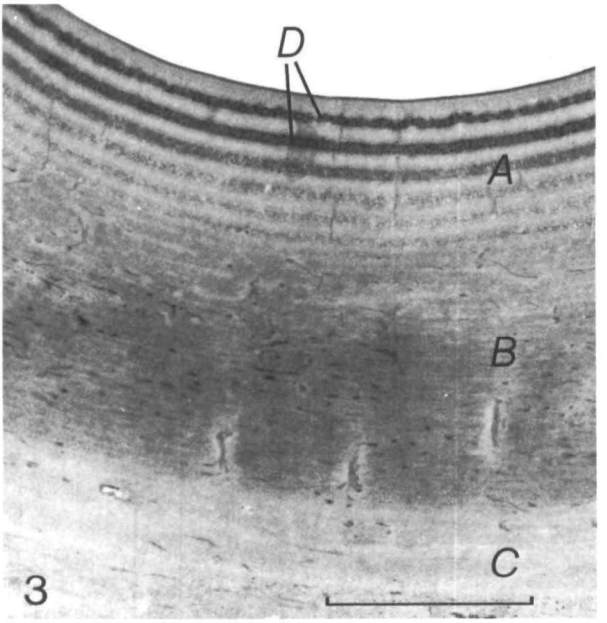

Fig. 3. Transmission electron micrograph of cross-section of elytral cuticle 12 h after ecdysis. The epicuticle (*A*) and outer exocuticle (*B*) are fully deposited. The inner exocuticle (*C*) is partially formed. The epicuticular *D* layers appear denser at this stage. Section was not post-stained. Scale bar, 1 μm.

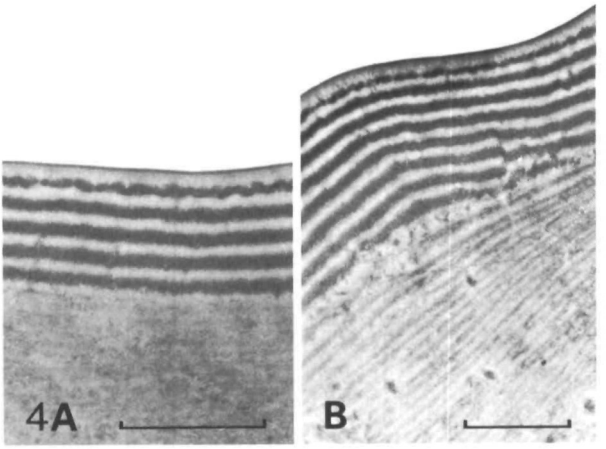

Fig. 4. Transmission electron micrograph of cross-sections of elytral cuticle 14 days after ecdysis. Epicuticular layers are fully developed and the electron density of the *D* layers is apparent in both unstained and post-stained sections. (A) Unstained; (B) post-stained. Scale bars, 1 μm.

of dense layers in the epicuticle (Fig. 4B), their high electron density is also apparent in micrographs of unstained sections (Fig. 4A).

DISCUSSION

The present study indicates that the potential cuticular reflector is formed prior to ecdysis, and therefore, cannot be considered as a dermal gland product. The early

deposition and location of the reflective layers indicate they are epicuticular. The first layer formed during a moult cycle is the cuticulin layer, followed by the inner epicuticle (Filshie, 1982). In pre-ecdysial samples of *C. scutellaris*, the potential reflective layers constitute the outer portion of newly formed cuticle, and display a thin, dense outer border, suggestive of a cuticulin layer. A partially deposited exocuticle appears below these layers and exhibits a helicoidal orientation of fibrils.

Micrographs of developing cuticle, and the continuity of dense layers produced by adjacent cells, indicate that a substrate or precursor is deposited during the initial formation of the epicuticle. The change in electron density of the dense layers suggests that a change in molecular chemistry occurs during the moult cycle. Either electron-dense material is deposited after formation of the epicuticle, or endogenous material within these layers is converted to compounds of higher electron density or affinity for OsO_4. Simultaneously, the colour of the newly emerged imago progresses from shorter to longer wavelengths, while dark pigmentation forms behind the reflecting layers. Although associated changes in refractive index cannot be determined, the increase in optical thickness of these layers must be responsible for the increasing wavelength of maximum reflectance observed during development.

In situ impregnations of developing cuticle have been reported previously. Wigglesworth (1976) described the spreading of a proteinaceous secretion within the epicuticle associated with sclerotization or melanization. It is generally assumed (Hackman, 1974) that a phenolic precursor of melanin is located in the integument at the site of pigmentation, perhaps as a by-product of sclerotization. This substrate is then assumed to be converted to melanin by an enzyme transported to this site *via* the pore canals.

Cicindelid pore canals contain materal substantially denser than the areas of exocuticle they traverse (Schultz & Rankin, 1985). Smaller extensions of the canals within the epicuticle possess electron-lucent lumina whose borders are dense. The dense contents of the pore canals may be substrates or enzymes dispersed in the exocuticle and epicuticle for sclerotization or pigmentation. If the dense epicuticular layers of cicindelids are melanoproteins formed *in situ*, the reaction entails a simultaneous increase in absorptivity and electron density.

These observations of colour development agree with those of Shelford (1917) and Willis (1967) for several *Cicindela* species. Shelford mistakenly assumed that the reflector was a single, thin layer containing materials with the properties of aniline dyes. He attributed the changing colours to changes in the chemical composition of the layer. While chemical characteristics of the reflector do appear to change during ecdysis, these changes occur within a multilayered ultrastructure that is formed during the initial stages of the adult moult.

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