

Juvenoids cause some insects to form composite cuticles¹

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SUMMARY

Metamorphosing insects treated with juvenoids may secrete composite cuticles which combine morphological features of two metamorphic stages within the area secreted by an individual epidermal cell. Characters found combined were pigmentation, tanning, surface sculpturing, and microtrichiae. Neighbouring cells frequently form different types of cuticle. Composite cuticles should not be confused with the more common mosaic cuticles, which are composed of discrete areas with different stage-specific morphology (e.g. some larval patches set in an otherwise normal adult cuticle).

Treatment of last instar nymphs of *Pyrrhocoris apterus* (Hemiptera) with a juvenoid induced secretion of composite cuticle which combined larval morphological features with imaginal pigmentation. Cells remained susceptible to composite cuticle induction over an extended period prior to the actual initiation of cuticle deposition.

Composite cuticle which contained larval microtrichiae and pupal tanning were induced with juvenoids in the lepidopterans *Hyponomeuta malinella*, *Hyphantria cunea*, *Spodoptera littoralis*, *Mamestra brassicae*, *Pieris brassicae*, *Aglais urticae*, but not in *Galleria mellonella*. Composite cuticle possessing both pupal and imaginal morphological features was produced with juvenoids in *Leptinotarsa decemlineata* (Coleoptera). It is suggested that this type of cuticle reveals the secretory capacities of a single epidermal cell when the cellular reprogramming from one developmental stage to the next has been stabilized at an intermediate point by juvenoids.

INTRODUCTION

That the action of juvenile hormone (JH) is not all-or-none at the level of an individual cell has until now been demonstrated in but two systems. Wigglesworth (1940, 1970) showed that *Rhodnius prolixus* exposed to JH at certain periods during the final larval instar produced cuticle, including bristles, which combined larval and adult characters. Similarly, Lawrence (1969) found that treatment of *Oncopeltus fasciatus* with a juvenoid between 49 and 74 h of the

¹ This study was dedicated to Professor Vincent Wigglesworth in appreciation of his discovery and recognition of the significance of composite cuticles forty-two years ago.

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150 h final instar resulted in cuticle with larval pigmentation and imaginal surface sculpturing. In these species, the combination of larval and imaginal characters can clearly be seen in tiny islands of cuticle secreted by individual cells, and both sculpturing and pigmentation may differ in immediately adjacent cells, i.e. each character is cell autonomous. Such cuticles have been called mosaic, but this term has also been used for cuticles of animals which have regions characteristic of different stages (see Willis, 1974, for review). We now introduce the term *composite cuticle* for cuticle produced by a single cell which combines features of two metamorphic stages. We suggest that the term *mosaic cuticle* be reserved to describe animals which combine two stages by having discrete areas with different stage-specific morphology (e.g. some larval patches set in an otherwise normal adult cuticle). The term mosaic has also been applied to moults initiated by sub-threshold doses of ecdysteroids (Truman, Riddiford & Safranek, 1974). Here only localized regions of the epidermis respond by forming a new cuticle. To avoid ambiguity, such animals could be described as having *regionally-replaced cuticle*.

These distinctions are essential in order to emphasize the significance of composite cuticle in evaluating the mode of action of JH. Hypotheses explaining the action of JH (Wigglesworth, 1970; Williams & Kafatos, 1972) have suggested that the genome is divided into stage-specific gene sets, and that JH prevents the use of a new gene set but permits the re-use of the set last used. If a single cell can secrete a cuticle with both larval and adult characters the simplicity of such hypotheses is compromised. Composite cuticles are consistent with a recent hypothesis that juvenile hormone acts on genetic reprogramming rather than at the level of stage-specific genes (Willis, 1981).

Sláma, Romaňuk & Šorm (1974) have questioned whether composite cuticles are sufficient to prove that JH does not act according to an 'all-or-none' rule at the cellular level, and claimed that such cuticles were probably a rare phenomenon, especially as they had not been observed in the extensively analysed species, *Pyrrhocoris apterus*. We now wish to report that composite cuticle is readily produced in *Pyrrhocoris*, as well as in insects belonging to two other orders, Lepidoptera and Coleoptera.

MATERIALS AND METHODS

The stock of *Pyrrhocoris apterus* L. was provided by Dr K. Sláma. The culture was maintained at 25 °C under a photoperiod of 16 h light and 8 h darkness. Freshly ecdysed nymphs of the fifth (final) larval instar were removed from the culture at least once a day and kept in separate containers. Under these conditions the fifth instar lasted 170–180 h. Larvae of the appropriate age were treated topically across the entire dorsal surface with the juvenoid methyl 7,11-dichloro-3,7,11-trimethyl-2-dodecenoate (Romaňuk, Sláma & Šorm, 1967). The compound was diluted in acetone and 2 µl of the selected concentration

applied topically (corresponding to 0.02, 0.2, 2 and 20 μg of the juvenoid per specimen). Treated nymphs were kept in paper-lined Petri dishes and provided with linden seeds and water. Animals were fixed in 70% ethanol 1–3 days after the next ecdysis.

The other species we examined came from earlier tests of various juvenoids (Sehnal, 1976). The lepidopteran larvae of *Spodoptera littoralis* (Boisd.) (Noctuidae), *Mamestra brassicae* L. (Noctuidae), *Pieris brassicae* L. (Pieridae) and *Aglais urticae* L. (Nymphalidae) were treated within the second quarter of the last instar, and those of *Galleria mellonella* L. (Pyrilidae), *Hyponomeuta malinella* Zell. (Hyponomeutidae), and *Hyphantria cunea* Drury (Arctiidae), within the third quarter of the last larval instar. In the case of the Coleopteran, the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Chrysomelidae), the juvenoids were applied on freshly ecdysed pupae. All species were fixed and stored in 70% ethanol within a few days after ecdysis following the treatment.

The cuticular characteristics were examined on the dorsal side of the fifth abdominal segment, except in *Leptinotarsa* where we have taken the fifth abdominal sternite. Excised pieces of the integument were thoroughly cleaned of adhering tissue and then boiled in saturated KOH for 3–10 min until the remaining cuticle became soft. The timing was crucial because excessive boiling bleached some of the black pigmentation. The processed cuticle was washed in water, dehydrated through ethanol and xylene and mounted in Canada balsam or Euparal. When shed exuvia were used, they were mounted without prior boiling in KOH. Such whole mounts of cuticle were examined with transmitted light, dark-field and phase-contrast optics.

Following such treatment it was possible to recognize an array of cuticular characters. We could usually distinguish between melanin pigmentation forming a surface pattern and the general cuticular tanning by sclerotins (Kayser-Wegmann, 1976). The other integumental pigments (pteridines, ommochromes, etc.) were lost during the processing. We could further distinguish several morphological characters: multicellular integumental structures (ridges); epidermal projections (macrotrichiae and their sockets); cuticular projections (microtrichiae and denticles); low surface sculpturing of the cuticle; and indications of the borders of the epidermal cells which had secreted the cuticle. In each species, these characters were examined in two to three cuticles of normal animals and in ten cuticles of the individuals which had been affected by the juvenoids. In a few cases the integument was freed of the adjacent tissue but the epidermis was left attached to the cuticle. These preparations were stained with Azure A (Himes & Moriber, 1956) to reveal the nuclei of the epidermal cells.

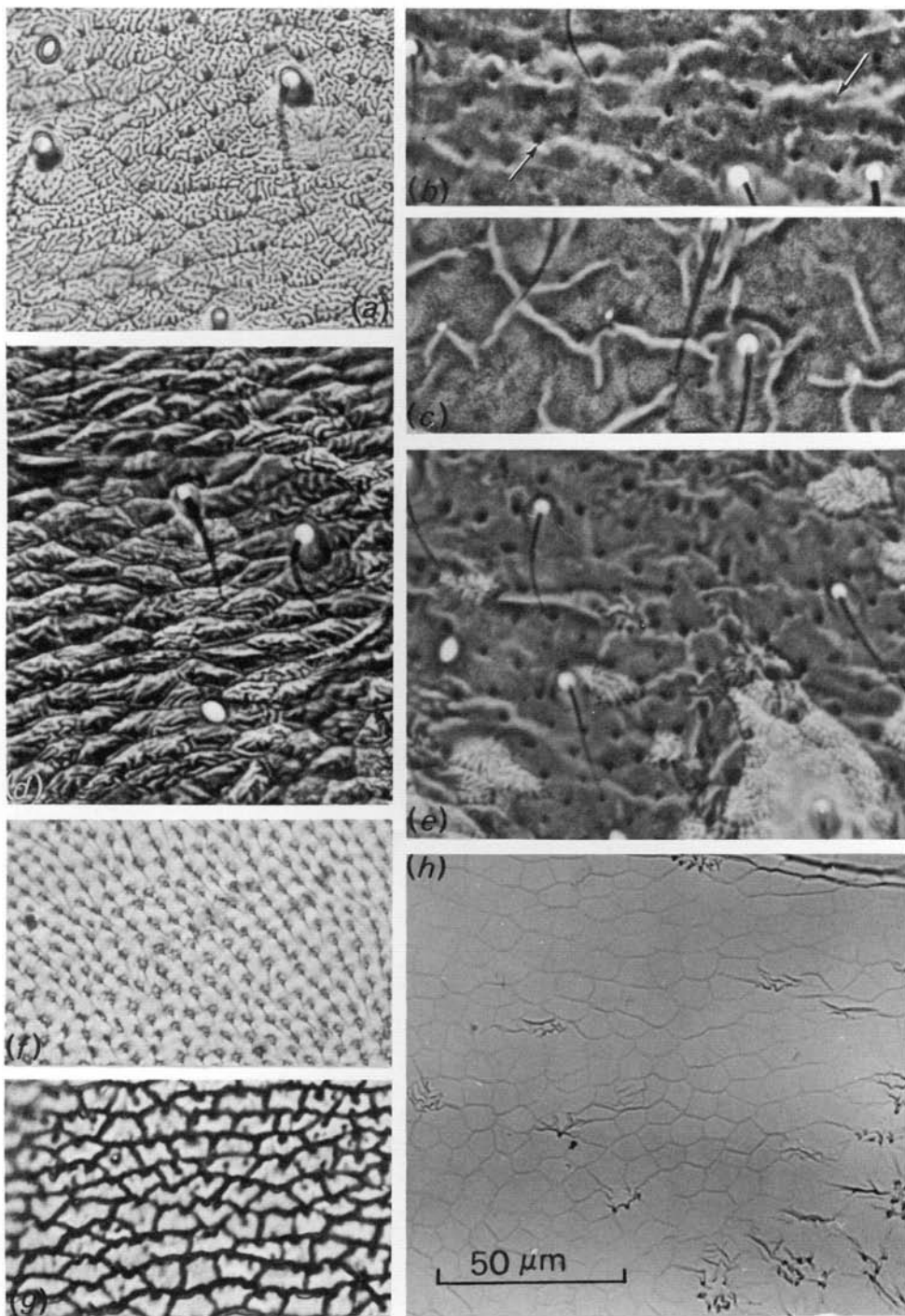


Table 1. *Character of the cuticle on the fifth tergite of Pyrrhocoris treated with the juvenoid at different ages of the last larval instar*

Juvenoid dose (μ g)	Time of treatment (h)	No. of bugs	% bugs with cuticle which is:			% bugs with dark or mixed cuticle which is, at least in part, composite
			Colourless (larval-like)	Mixed	Dark (adult-like)	
20-00	5-21	5	100	0	0	0
20-00	45-52	7	45	55	0	30
20-00	54-69	5	40	60	0	100
20-00	77-92	10	10	60	30	100
20-00	123-147	9	0	0	100	0
0-02	5-21	10	0	100	0	50
0-02	29-45	8	0	75	25	0
0-02	54-69	10	0	40	60	0
0-02	77-92	10	0	10	90	0

RESULTS

Larval-adult composite cuticle in Pyrrhocoris apterus

Characteristics scored. Four separate cuticular features which change during metamorphosis can be distinguished on the fifth abdominal tergite: pigmentation, cuticular projections, surface sculpturing, and imprints of cellular boundaries. The larval cuticle is colourless except for a black area around each of the stink gland openings. The entire surface of the tergite is covered with tiny dots, arranged in about five rippled rows within each cell territory (Fig. 1a). In contrast, the imaginal cuticle is dark brown, and shows no surface sculpturing or imprints of cell outlines across the entire segment (Figs. 1b, c). There are, however, several rows of backward-pointing denticles adjacent to the anterior margin of the tergite (Fig. 1b).

Effects of the juvenoid. The gross morphological effects of the treatments were the same as described by Williams & Sláma (1966) but microscopic examination of the cleaned cuticles revealed abnormalities in insects which appeared to the

Fig. 1. Surface views of cuticles. (a-e) Cuticles from the linden bug, *P. apterus*. (a) Transparent larval cuticle from anterior margin of segment with rippled surface pattern and dense areas in some cellular territories. (b) Imaginal cuticle from the anterior segment margin, note denticles (arrows). (c) Imaginal cuticle from the central region of segment showing territorial lines. (d) Composite cuticle combining larval cell boundaries and remnants of ripples with imaginal pigmentation. (e) Unicellular and larger islands of composite cuticle in an imaginal area adjacent to larval cuticle (bottom, right). (f-h) Cuticles of the ermine moth, *H. malinella*. (f) Hairy larval cuticle. (g) Pupal cuticle with distinct cell boundaries. (h) Composite cuticle. Note graded preservation of larval features.

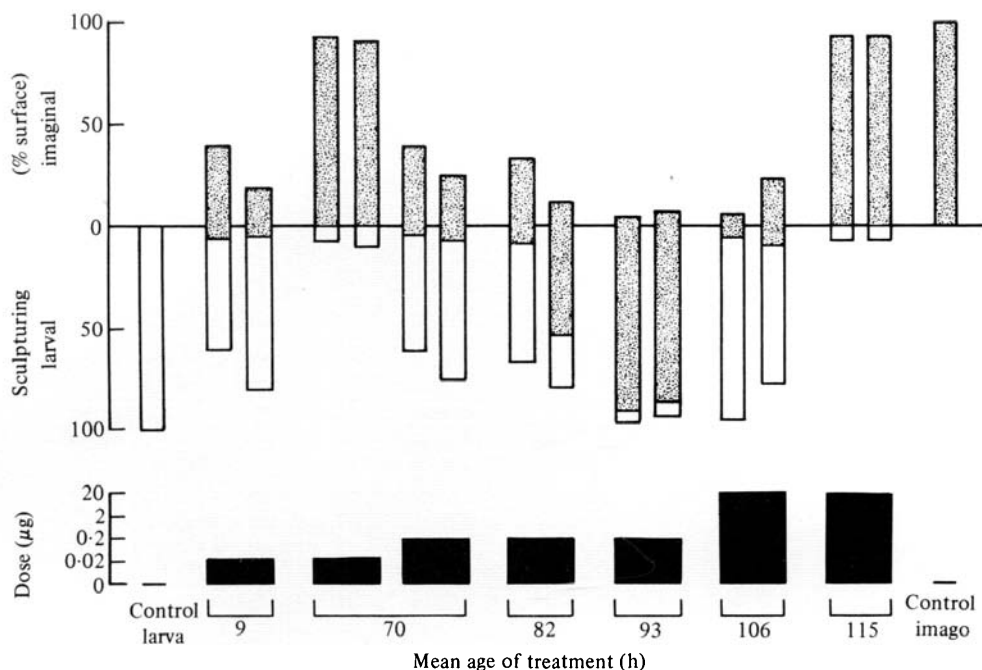


Fig. 2. Distribution of different types of cuticle on the fifth abdominal tergite of *Pyrrhocoris* following various treatments with a juvenoid. Each upper bar represents a single individual, dotted areas indicate pigmented cuticle. The amount of composite cuticle in an individual is represented by the dotted area below the zero line where imaginal pigmentation is combined with larval sculpturing. Doses of the juvenoid (in $\mu\text{g}/\text{specimen}$) are indicated by the lower dark bars. Time of treatment is given in hours of the last larval instar and is mean time $\pm 10\%$, except for the youngest group where the animals ranged in age from 1–18 h.

naked eye as normal adults. The results of juvenoid application to last instar larvae of various ages are shown in Table 1. Here two independent observers scored cuticles first by the criterion of colour and then by the presence of composite cuticle. Most of the mixed cuticles could also be classified as mosaic as they bore patches of typical larval and typical imaginal cuticle. However, many also had regions of composite cuticle, where brown (imaginal) pigmentation was combined with larval sculpturing. Importantly, some animals with uniformly adult-like pigmentation had areas of composite cuticle. Tergites with mixed pigmentation were obtained with 0.02 – $20 \mu\text{g}$ of the juvenoid applied up to 92 h of the last instar. The highest dose of $20 \mu\text{g}$ induced mosaics also when applied at 101 h (not in the Table). It is obvious that with the high doses mosaic and/or composite cuticle prevailed after late treatment (45–92 h), whereas with the lowest dose they dominated after early treatment (5–45 h).

Figure 2 shows the proportion of different types of cuticle on representative individuals. These proportions varied and depended on both the time of treatment and the dose. For example, there were individuals treated with $0.02 \mu\text{g}$

at 70 h or with 0.2 μg at 93 h which had about the same fraction of the tergite pigmented. Yet, structurally, most of the brown regions were perfectly imaginal in the former and composite in the latter.

Character of the composite cuticle. Several classes of composite cuticle, clearly produced by individual cells, were recognized. Most common was pigmented cuticle (an imaginal character) with cellular boundaries and rippled surface sculpturing (larval characters) (Fig. 1*d*). Such areas were often extensive, covering most of the tergite. In some cases the composite cuticle lacked the rippled pattern, but had well-defined cellular territories. In some specimens we found cuticle near the anterior tergite border which had combined the imaginal characters of denticles and pigmentation with cellular boundaries and surface ripples. None of the composite cuticles contained the clear ridges which divide the normal adult cuticle into distinct multicellular regions (Fig. 1*c*).

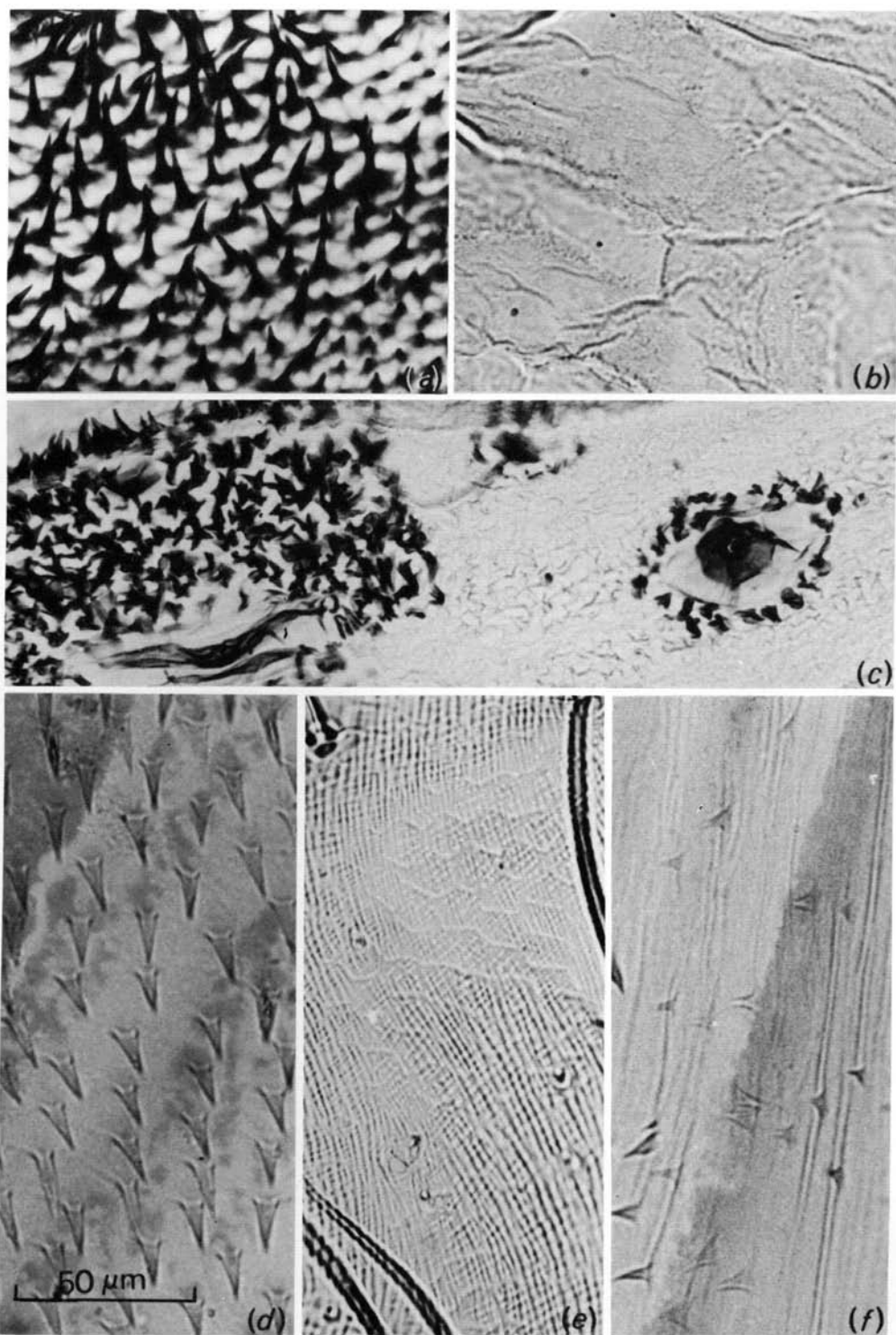
Distribution of composite cuticle was irregular. In some instances it was produced by only one or a few adjacent cells in an area of either perfectly larval or perfectly imaginal cuticle (Fig. 1*e*). Isolated pigmented patches of sculptured (composite) or unsculptured (normal imaginal) cuticle in large regions of colourless larval cuticle were abundant in animals treated with low doses prior to 45 h, or with high doses as late as 105 h. Even when much of the surface was covered with composite cuticle, one could find that an occasional cell had formed pigmented and smooth cuticle.

Larval-pupal composite cuticles in Lepidoptera

Hyponomeuta malinella. Except for eight macrotrichiae, the ermine moth's larval cuticle on the dorsal side of the fifth abdominal segment is uniform. It is densely covered with microtrichiae, which are 8–20 μm long and set 5–10 μm apart (Fig. 1*f*). Most of them have a long narrow transparent shaft but those in the dark pigmented regions on the lateral parts of the segment are dark, short and broad, and thorn-like. The cell borders cannot be recognized, but counts of the nuclei of epidermal cells have indicated that each cell produces 4–14 microtrichiae.

The cuticle of ermine moth pupae is tanned to light brown across the entire segment, lacks microtrichiae, and its surface is broken into fields corresponding to individual epidermal cells (Fig. 1*g*). At regular distances the integument forms multicellular ridges which delineate areas corresponding to 150–200 epidermal cells.

There was usually a sharp line between regions of larval and pupal cuticle in the larval-pupal intermediates we have examined, but some larval-like areas contained abnormal larval bristles, which were deformed and reduced in size. Areas of cuticle with clearly composite characters were found in all individuals we examined. The composite cuticle resembled pupal cuticle by being slightly tanned but it lacked the multicellular integumental ridges and the cell boundaries were less distinct than in pupae. Often it contained islands of 1–20 larval



microtrichiae reduced to various degrees (Fig. 1*h*). They seemed to be produced predominantly at the points of contact between adjacent epidermal cells. Their size ranged from a few microns to mere wrinkles on the cuticular surface. Some of the better preserved microtrichiae were pigmented as in larvae.

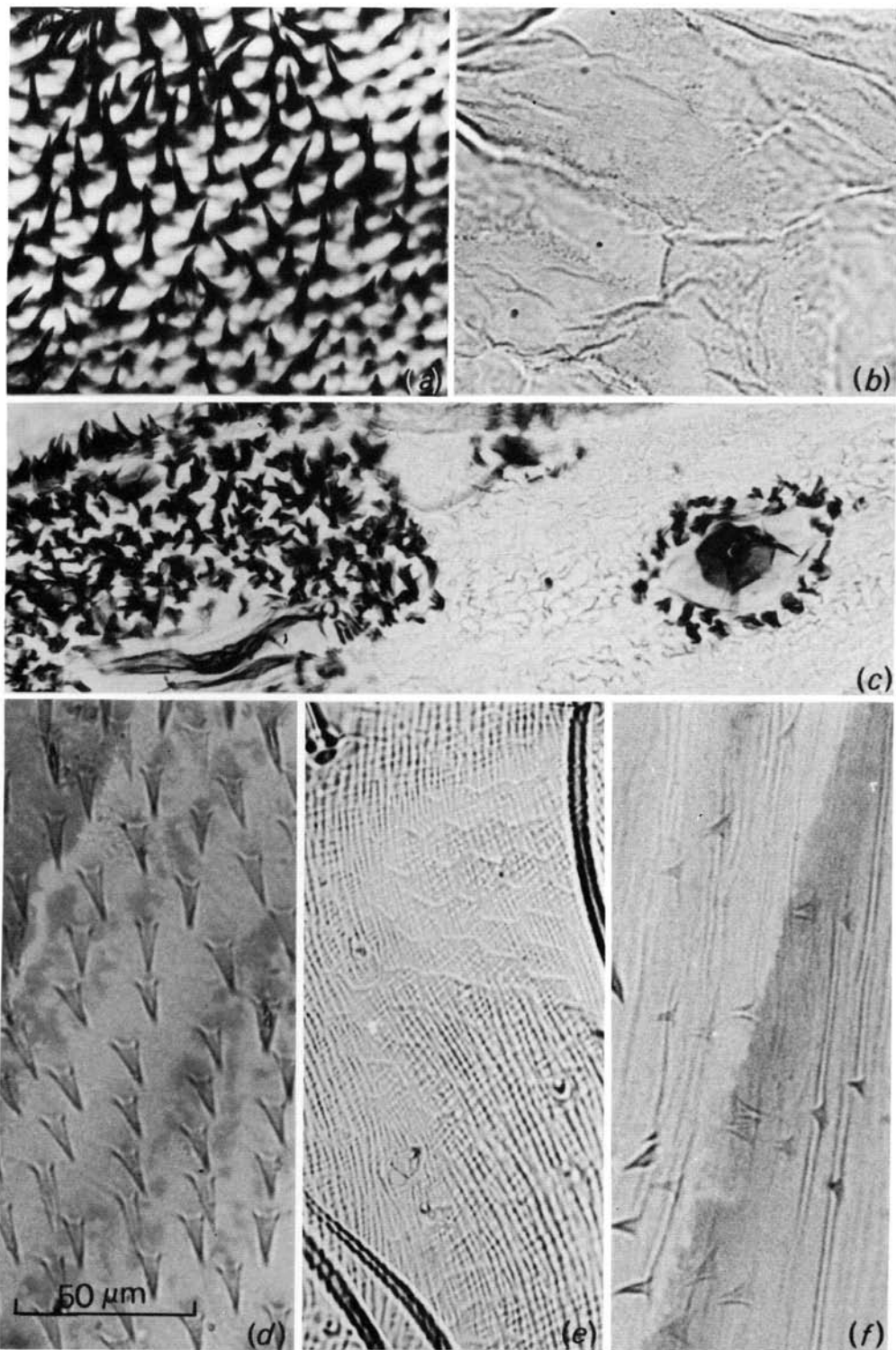
Hyphantria cunea. In fall webworm larvae, the dorsal part of the fifth abdominal segment contains four large circular plaques and a few small spots of brown tanned cuticle. The plaques bear long melanized macrotrichiae with lighter sockets and numerous evenly distributed microtrichiae. The cuticle between the tanned plaques is uniformly transparent and soft and covered with microtrichiae. Each larval epidermal cell produces a group of two to eight (usually four) thorn-like microtrichiae of conical shape (Fig. 3*a*). The base of the thorns often contains black pigment which is responsible for the dark coloration of much of the dorsal side of the caterpillar. In some regions the microtrichiae are not grouped but set apart in more or less equal spacing. The cell boundaries are barely perceivable in some regions of larval cuticle.

The pupal cuticle does not bear any thorns or bristles and is tanned uniformly brown across the entire segment. At regular distances of 8–20 cells the pupal integument forms conical multicellular pits. The cell boundaries of the pupal cuticle are more distinct than in the larvae (Fig. 3*b*).

The larval–pupal intermediates occasionally possessed well-outlined regions of perfectly larval or perfectly pupal cuticle. The plaques of tanned larval cuticle were reduced in size and contained fewer and smaller macrotrichiae, which often lacked the larval melanin. In many specimens these plaques were broken into smaller fields. The regions between the plaques were a complex mixture of cuticle morphology. The composite character was indicated by the reduction of microtrichiae in the larval-like areas of untanned cuticle or by the presence of microtrichiae in pupal-like tanned cuticle (Fig. 3*c*). The number of microtrichiae produced by one cell was often reduced to one to three and their size ranged from nearly normal to tiny elevations of the cuticle. Variation in the formation of microtrichiae was enormous and seemed independent of the presence or absence of pupal tanning. It was difficult to find two adjacent cells with exactly the same type of cuticle (Fig. 3*d*). Some microtrichiae in both tanned and untanned cuticular regions contained the black larval pigment.

Spodoptera littoralis and *Mamestra brassicae*. Except for a few melanized macrotrichiae, the larval cuticle of both the Egyptian cotton leafworm and the

Fig. 3. Surface views of Lepidopteran cuticles. (*a–d*) Cuticles of the fall webworm, *H. cunea*. (*a*) Larval cuticle with rosettes of thorns produced by individual epidermal cells. (*b*) Pupal cuticle with faint cell boundaries. (*c*) Uniform region of composite cuticle. (*d*) Area with unicellular islands of composite cuticle of variable composition. (*e–g*). Cuticles of the cotton leafworm, *S. littoralis*. (*e*) Larval cuticle with uniform distribution of dots. (*f*) Pupal cuticle, tanned with cell boundaries. (*g*) Composite cuticle showing wide variation in expression of larval characters by individual cells.



cabbage armyworm is smooth. It is densely dotted with what appear to be tiny indentions of about $1\text{ }\mu\text{m}$ in diameter and the same distance apart (Fig. 3e). The cleaned larval cuticle of *Spodoptera* is transparent in some areas and grey in others; that of *Mamestra* is irregularly brown. The cell boundaries cannot be recognized.

The pupal cuticle of both species is tanned brown and shows faint imprints of cell boundaries (Fig. 3f). These are transparent and very distinct in the intersegmental region. The tergite contains but four short and transparent bristles and numerous multicellular conical depressions of the integument. A narrow band between the sclerite and the intersegmental part is decked with tiny backwards-pointing denticles.

The larval-pupal intermediates of both species showed areas of larval, pupal and composite cuticles. The macrotrichiae were usually of a size intermediate between larval and pupal and devoid of melanin. The composite cuticle was in some cases soft as in larvae but the surface indentions were unclear and sparsely distributed; larval pigmentation was often preserved in *Spodoptera*, rarely in *Mamestra*. In the more convincing cases the composite cuticle possessed pupal tanning and contained scattered islands of larval surface sculpturing (Fig. 3g). Such cuticle occurred anywhere on the tergite, including the integumental pits, but cell boundaries were obvious only in the intersegmental region. The islands of larval sculpturing apparently corresponded to from one to many epidermal cells (diameter of the smallest islands was less than $10\text{ }\mu\text{m}$). In extreme cases the sculpturing was reduced to slight granulation of the tanned cuticular surface but mostly it had the normal larval appearance.

Pieris brassicae. The fifth abdominal segment of the cabbage white caterpillar bears on the dorsal side more or less uniformly distributed macrotrichiae of various sizes. Most of them contain melanin. Some other macrotrichiae are arranged in two large brown and smooth circular regions in the posterolateral corners of the dorsal side. The remaining cuticle is uniformly covered with thorn-like microtrichiae (Fig. 4a). At its base each thorn diverges into several crests which give a stellate appearance when viewed in the ground plane. Most thorns have a wide black base but some are narrow, long and transparent. In a few places several thorns are grouped to form tanned rosettes. The diversity of thorns in size and pigmentation creates the surface patterning of the *Pieris* caterpillar.

Fig. 4. Surface views of cuticles. (a-c) Cuticles of the cabbage white butterfly, *P. brassicae*. (a) Larval cuticle with melanized thorns. (b) Pupal cuticle, smooth except for fine granulations. (c) Composite cuticle with reduced thorns, some melanized, some not. (d-f) Cuticles of the Colorado potato beetle, *L. decemlineata*. (d) Pupal cuticle with fine spikes. (e) Imaginal cuticle with faint cell boundaries and striated surface. (f) Composite cuticle characterized by incomplete development or lack of both pupal and imaginal features.

The pupal cuticle of *Pieris* contains evenly distributed small and transparent macrotrichiae, is gently wrinkled and shows very fine dots on the surface (Fig. 4b). It becomes transparent after boiling with KOH, except for ten large oval plaques and some bristle sockets which remain dark brown.

The larval-pupal intermediates contained large regions of cuticle which could be characterized as composite. The composites ranged from larval-like cuticle with slightly reduced thorns to pupal-like cuticle which showed remnants of larval thorns as tiny cuticular elevations (Fig. 4c). There was an uninterrupted series of cuticular types between these two extremes. Thorns which were reduced to about a third of the normal size (and concurrently deformed in shape) contained the black larval pigment, whereas the more suppressed thorns were devoid of it. In some regions the cuticular surface was corrugated and no typical larval or pupal features could be recognized (Fig. 4c). The corrugation may represent a transient form between the larval and pupal cuticles. The large macrotrichiae which are apparently descendants of those found in larvae were usually of intermediate size and lacked the larval pigment. The brown tanning around the bristles varied in intensity.

The larval, pupal and composite cuticles of *Aglais urticae* were similar to those described for *Pieris*.

Galleria mellonella. The cuticle of all developmental stages of the wax moth has been described by Heims (1956). In the larval-pupal intermediates produced with juvenoids, larval and pupal cuticle occurred on the same animal but retained their typical character. Cuticle secreted by a single cell which combined larval and pupal features was not found. The only possible indication of a composite character was the reduced size of exocuticular structures in larval cuticle which was immediately adjacent to pupal cuticle.

Pupal-imaginal composite cuticle in Leptinotarsa decemlineata

The processed pupal cuticle of the Colorado potato beetle is light brown in the sternite and transparent in the intersegmental region. The brown tanning is intense in small spots scattered towards the sides of the segment. The cuticle shows no cell boundaries and is covered with spike-like denticles which are tiny and widely distributed in the central part but dense and 10 μm long in the intersegmental region (Fig. 4d).

The imaginal cuticle lacks the denticles, shows borders of epidermal cells, and its surface is delicately grooved with parallel rows of tiny dots (Fig. 4e). An oblique black area with very distinct cell boundaries is found in each posterior corner of the sternite. Slender macrotrichiae are more or less evenly distributed across the entire sternite.

The pupal-imaginal intermediates rarely possessed cuticle which was perfectly pupal or perfectly imaginal. Most cuticle lacked all pupal features but only some of the adult features were developed. Frequently, imaginal macrotrichiae, often reduced in number, were present on cuticle without any pigmentation or surface

Table 2. Characteristics of typical composite cuticles found on the fifth abdominal tergite after treatments with juvenoids

Species	Character				
	Melanin pigmentation	Tanning	Tubercles, microtrichiae, denticles	Surface sculpturing	Imprints of cellular boundaries
<i>Oncopeltus</i>	L ⁺	—	I ⁽⁺⁾	L ⁻	L ⁺
<i>Pyrrhocoris</i>	I ⁺	—	I ⁽⁺⁾	L ⁽⁺⁾	L ⁺
<i>Hyponomeuta</i> and <i>Hyphantria</i>	L ⁺	P ⁺	L ⁽⁺⁾	—	P ⁺
<i>Spodoptera</i> and <i>Mamestra</i>	L ⁺	P ⁺	—	L ⁽⁺⁾	P ⁽⁺⁾
<i>Pieris</i> and <i>Aglais</i>	L ⁽⁺⁾	P ⁽⁺⁾	L ⁽⁺⁾	P ⁻	—
<i>Leptinotarsa</i> (5th abd. sternite)	I ⁺	P ⁻	P ⁽⁺⁾	I ⁽⁺⁾	I ⁽⁺⁾

Listed characters are typical for the larval (L), pupal (P), and imaginal (I) cuticles. +, presence; —, absence and (+) development to varying degree of a character. Melanin pigmentation was scored in regions where it occurs in normal pattern of only one stage. Data for *Oncopeltus* were taken from Lawrence (1969) and Willis & Hollowell (1976).

sculpturing. Less often the cuticle bore scattered pupal thorns which were reduced in size (Fig. 4f). In one out of the ten examined animals we found the two large areas of black pigmentation which are typical of imagoes, but imaginal surface sculpturing was not developed in this region at all. A very rare but most clearly composite cuticle occurred in small patches which contained reduced pupal denticles in combination with imaginal grooves and cell boundaries.

DISCUSSION

Characteristics of composite cuticle

Insects whose metamorphosis is partially inhibited by juvenoids display a variety of characters which are intermediate between two developmental stages. Most of these characters are based on differential responses of groups of cells to the hormone, forming a mosaic pattern. The composite cuticles we have described exhibit characteristics of two developmental stages in cuticle secreted by a single cell.

In order to understand the basis for such composites it is essential to establish that each of the characters is cell autonomous. One must rule out the possibility that a character (e.g. pigmentation) is dependent on the precursors to which the cell is exposed rather than its own developmental stage. In each of the species described in this paper, we have found composite cuticles in islands comprising from one to a few cells surrounded by cuticle with all the normal characteristics of one of the developmental stages. By this criterion, pigmentation, cuticular projections (microtrichiae, denticles), surface sculpturing, and distinct cell boundaries are cell-autonomous characters.

In Table 2 we show that different, but precise, combinations of these characters appear in different species. In both Heteroptera and Lepidoptera, composite cuticle typically possesses the pigment of one developmental stage and the surface pattern of the other stage. In *Leptinotarsa* the composite nature of the cuticle lies either in combination of its pupal and imaginal morphological features or simply in incomplete development of imaginal characters.

Deviations of microtrichiae and surface sculpturing from the normal size might also be an indication of the composite nature of the cuticle, although a true combination of characters of two developmental stages occurs only within a certain range of size variation. For example, the composite cuticle of *Oncopeltus* displayed larval pigment only when imaginal tubercles were very poorly developed; when their numbers were less reduced, the pigment was absent (Lawrence, 1969). By contrast, pupal tanning in the lepidopterans we studied was sometimes combined with nearly fully developed larval morphological features.

A special case of composite cuticle was found in epidermal projections such as bristles and sockets which normally either change their shape or degenerate during metamorphosis. The specimens treated with juvenoids bore projections which were intermediate between their appearance in the two developmental stages. Intermediate bristles were found by Wigglesworth (1934) in *Rhodnius*, by Piepho (1942) in *Galleria*, by Lawrence (1969) in *Oncopeltus*, and in all the lepidopterans examined in this study.

Finally it should be noted that cuticle with a particular mixture of features can reflect a juvenoid induced abnormality for one region and normal morphology for another. Thus in *Pyrrhocoris* we have found cuticle surrounding the normal larval stink gland opening which resembles composite cuticle in having dark pigmentation combined with ripples and cellular boundaries. This combination of features is normal for that particular larval region. Lawrence (1969) described a comparable situation in *Oncopeltus*. It has also been found that different regions of the same segment may have different cuticular proteins (Willis, Regier & Debrunner, 1981) and differential sensitivity to hormones (Mitsui & Riddiford, 1976).

The effect of timing and dosage of juvenoids on composite cuticle formation

In this study the lepidopterans and the Colorado potato beetle were treated with juvenoids in carefully selected periods when small alterations of the dose caused great difference in the response (Sehnal, 1976). Composite cuticle was found in individuals which had been produced with juvenoid concentrations varying over several orders of magnitude. In *Pyrrhocoris*, also, composite cuticles were formed with doses ranging from 0.02 to 20 μg .

We carried out an extensive analysis of the responsiveness of *Pyrrhocoris* to juvenoid application. At the onset of the instar, high doses are more effective than low, presumably because they persist longer or activate the animal's own

corpora allata. Subsequently one finds that loss of sensitivity is dose dependent, for the cells become insensitive to increasingly higher and higher juvenoid doses until shortly before cuticle secretion commences, when they become insensitive to any dose of juvenoid; comparable findings have been reported for *Galleria* (Sehnal & Schneiderman, 1973). Data in Fig. 2 demonstrate that with a suitable juvenoid dose one can induce formation of composite cuticle throughout this period. Thus commitment to the secretion of perfectly imaginal cuticle does not occur until just prior to cuticle secretion.

Production of composite cuticle proves that at certain times sensitivity to juvenoids differs for various functions within a single cell. Linkage in the occurrence of certain characters indicates that their sensitivity to juvenoids was similar. For example, in *Pyrrhocoris* larval cellular boundaries were found associated either with the normal larval rippled pattern or with less regularly arranged granules. These features could occur either in the presence or absence of pigmentation or denticles. Whenever cells near the anterior margin of the segment had formed cuticle with imaginal pigmentation, imaginal denticles were also present. Territorial lines, an imaginal character, were present only when all larval characters had been suppressed.

Significance of composite cuticle

The period from apolysis to ecdysis generally occurs more rapidly in the presence of juvenoids than in a normal metamorphic moult. Could this acceleration account for some of the features of composite cuticles, especially the reduction in size of microtrichiae and denticles? Probably not, for, in all species, cuticle formation and later (after ecdysis) pigmentation commence nearly simultaneously all over the abdomen. A precocious interruption of these processes would not be expected to result in the small patches of composite cuticle observed in treated specimens but rather in an over-all uniform effect.

The dependence of the localized secretion of composite cuticle on the dose and timing of juvenoid treatment must indicate that the character of the cuticle reflects the degree of commitment of the respective epidermal cells which was stabilized by the juvenoid at a transient point of cellular reprogramming. It would be premature to speculate as to the molecular level at which this reprogramming occurs. We know nothing about the molecules which underlie the different cuticular configurations nor even whether the initial molecular events which start a cell along a particular pathway of pigmentation or sculpturing are enacted simultaneously or sequentially. We merely wish this paper to emphasize that the action of juvenile hormone is not all-or-none at the level of a single cell; to the contrary, the reprogramming of cuticular synthesis and secretion may be inhibited at two or more levels. Insect epidermis provides a unique system in which to study such effects of hormones on a sequence of events within a single cell. It is difficult to imagine comparable studies in vertebrates where tissue heterogeneities preclude definitive knowledge of what a single cell is doing.

We thank Dr Karel Sláma for the impetus to carry out this study, for the stock of *Pyrrhocoris*, and for his comments on the manuscript. This work was begun while J.H.W. was an Exchange Scientist of the U.S. National Academy of Sciences and the Czechoslovak Academy of Sciences; additional support came from her grant AG-00248 from the National Institutes of Health. R.R. was a holder of a Postgraduate Scholarship of the Czechoslovak Ministry of Education.

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(Received 24 August 1981, revised 29 April 1982)