Morphology of *Ceroplastes pseudoceriferus* GREEN with Special Reference to the Wax Secretion

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Morphological observations on *Ceroplastes pseudoceriferus* GREEN, one of the common scale insects in Japan, were carried out throughout the postembryonic development with a special interest on the relations of the dermal gland pores and ducts to the wax secretion. The progress of morphological changes during the development from the first instar larva to the adult was described in detail, with reference to the external appearance of the waxy covering of each instar. The dermal gland pores and ducts could be classified into six types: Simple pore, cruciform pore, quinquelocular disc pore, multilocular disc pore, tubular duct and filamentous duct. On the basis of the distribution and the time of appearance on the body surface it is inferred that the simple pore is responsible for the secretion of wet wax which is produced after the third instar, the quinquelocular disc pore for powdery wax in the stigmatic furrows, and the multilocular disc pore for powdery wax around the vulva. By the method used, no type of pores or ducts which are responsible for the secretion of dry wax in the first and second instars, was found.

INTRODUCTION

The genus *Ceroplastes* is a large group, comprising many species in every biogeographical region, and is especially well represented in the tropics, subtropics and other warm parts of the world. There are three species of the genus *Ceroplastes* in Japan, *C. rubens* MASKELL, *C. japonicus* GREEN, and *C. pseudoceriferus* GREEN.¹

C. pseudoceriferus is widely distributed in Japan, Formosa, India and Ceylon, and an important pest of various fruit-trees and other cultivated plants. Because of the thick, dense waxy body protection covering this scale insect, its insecticidal control remains so far difficult.

This covering consists of waxy substance extractable with chloroform and aqueous substance designated as interior honeydew (TAMAKI and KAWAI, 1966).

¹ In Japan, *C. ceriferus* and *C. floridensis* were used generally for *C. pseudoceriferus* and *C. japonicus*, respectively (TAKAHASHI, 1953).

The present paper deals with the morphology of *C. pseudoceriferus* during the growth, with a special interest in distribution of gland pores and ducts in relation to wax secretion.

MATERIALS AND METHODS

Adult females were collected from twigs of tea plants in Tokyo about the middle of June, when they were in oviposition. Eggs were inoculated on fruits of a pumpkin variety, Hyuga (*Cucurbita moschata* DUCH.). Crawlers appeared at the end of June and settled on the whole surface of the pumpkin fruits. Rearing was carried out under room temperature. There was no remarkable difference between the laboratory rearings and the field observations in the growth of the scale insect.

Appropriate number of individuals were collected at random from the pumpkin fruit every 5 to 10 days. After observation of the waxy covering under a binocular microscope, these individuals were placed in a small crucible containing about 5 ml of 10% KOH, and gently heated for about 5 min on a hot plate. The specimens were then transferred to a small crystal dish containing distilled water, and, if necessary, remaining body contents were removed by a fine needle from a rupture made on the abdominal margin. The specimens were heated in 4-5 ml Essig's aphid fluid, a lactophenol solution (ESSIG, 1948), until they become transparent, and then washed with distilled water for about 10 min. After neutralization with acetic acid, the insect bodies were stained with acid fuchsin in a hydrochloric acid solution (Gage's acid fuchsin). They were dehydrated with 95% ethyl alcohol, cleared in phenolxylene (1:4) for 30 min, and then mounted on glass slides with Canada balsam. The specimens thus prepared were observed under a microscope. The body size, anal tubercles and anal plate were measured by a microscopic micrometer.

GENERAL MORPHOLOGY

Body

First instar (Fig. 2·A): Elongate oval and flat in form. Derm membranous. Spiracular clefts narrowly incised. Eyes on margin of head, small; similar through all succeeding instars. Mouth-parts and spiracles of types which are common to other Coccidae. Anal cleft rather short with large marginal seta on each caudal angle. Marginal setae small and fine; about 13 on each side. Submarginal and ventral setae minute, fine, smaller than marginal setae, similar in all succeeding instars.

Second instar (Fig. 3·A): Oval and flat in form. Derm membranous. Spiracular clefts narrowly incised. Anal cleft deeply incised with 2 large marginal setae on each caudal angle; numerous derm granulations along anal cleft. Marginal setae short and fine, about 14 on each side.

Third instar (Fig. 4·A): Broadly oval, rather convex dorsally. Derm membranous. Spiracular clefts wide, slightly concave. Anal cleft deeply incised with 3 to 4 large marginal setae on each caudal angle; numerous derm granulations

along anal cleft. Marginal setae minute and fine, about 15 on each side.

Fourth instar (Fig. 5·A): Broadly oval or almost circular, convex dorsally, hemispherical and sclerotized at maturity. Spiracular clefts broad and concave. Vulva opposite anal plates in a transverse slit. Anal cleft deeply incised with 4 to 6 large marginal setae on each caudal angle; numerous derm granulations along anal cleft and around vulva. Marginal setae small, rather stout, about 18 on each side; many small dorsal setae apically truncated or bluntly pointed on whole surface of dorsum. Some translucent pores just above base of anal plates. Circumference of anal plates heavily sclerotized, forming into a tubercle at maturity (Fig. 5·C); the tubercle almost conical in shape and variable in size, over 1 mm at maximum in length, apically with anal plates protruding beyond waxy covering.

The ranges of body length and width of each instar are shown in Table 1.

Table 1. RANGES OF BODY LENGTH AND WIDTH IN EACH INSTAR OF *Ceroplastes pseudoceriferus* GREEN REARED ON PUMPKIN FRUIT

Instar	Body length (μ)		Body width (μ)	
	Max.	Min.	Max.	Min.
I	700	365	440	205
II	990	640	725	420
III	1,963	738	1,525	625
IV	4,550	1,263	4,450	1,138

Antennae (Fig. 2·B)

Six-segmented; size not so variable through all instars; segment 1 robust; segment 2 with one small clear spot which is probably identical with structure named 'sensorium'; segment 3 elongate; segments 4 and 5 with one fleshy seta; segment 6 with 3 similar setae; each segment with a few hair-like setae, those on terminal segment long and particularly conspicuous in first instar.

First instar (Fig. 2·B1): Segment 3 almost equal in length to segments 4 and 5 together.

Second instar (Fig. 2·B2): Segment 3 shorter in length than segments 4 to 6 together.

Third instar (Fig. 2·B3): Segment 3 equal in length to segments 4 to 6 together.

Fourth instar (Fig. 2·B4): Segment 3 distinctly longer in length than segments 4 to 6 together.

Legs (Fig. 4·C)

Three pairs of legs similar to each other, well developed, slender and prominent in instar 1, but gradually reduced in size in comparison with body in succeeding instars. Trochanter with two sensoria on each face. Tarsus with one pair of slender tarsal digitules, one of which is rather thin in instars 1 and 2. Tibio-

tarsal articulation without condyle, tarsus apparently not movable. One pair of ungual digitules rather short, one of them stout, other comparatively slender.

Spiracular Setae (Figs. 2·A, 3·A, 3·A, 4·A, 5·B)

First and second instars (Figs. 2·A, 3·A): Three setae on each spiracular cleft on dorsal side; middle seta conical, bluntly pointed, and longer; other two setae globular or conical.

Third instar (Fig. 4·A): One large and 2 rather small, conical and bluntly pointed setae on dorsal side and one single row of 6 to 10 small, conical and sharply pointed setae on ventral side of each cleft.

Fourth instar (Fig. 5·B): Three to 5 large and 40 to 50 small, conical, bluntly or sharply pointed setae crowded in a cluster on margin of each spiracular cleft.

Anus (Fig. 4·B)

Structurally similar in all instars. Anal ring sclerotized, with 6 very large and 2 comparatively small setae, and with many translucent pores which are surrounded by one strongly sclerotized rim. Anal tube cylindrical, invaginated, membranous, with many longitudinal dermal folds. Anal ring freely movable in and out of anal tube.

Anal Plates (Fig. 2·C)

First instar (Fig. 2·C1): Almost triangular, rounded apically; lateroposterior margin almost straight or somewhat concave and a little shorter than lateroanterior margin. Three stout and one long apical setae on distal part, the long seta about 4 times as long as anal plate; one small and tubercular discal seta at about apical one-third; one fringe seta on each side.

Second and third instar (Fig. 2·C2, C3): Stout, almost triangular; lateroposterior margin slightly convex and nearly as long as lateroanterior margin. One rather small and 2 long stout apical setae on apex to distal half of mesal side;

Fig. 1. Anal plate length in each instar. Numbers of individuals examined are as follows: 1st instar, 41; 2nd instar, 46; 3rd instar, 133; 4th instar, 30.

one long stout discal seta at about apical one-fourth; 2 fringe setae on each side.

Fourth instar (Fig. 2·C4): Stout, roundish and almost semicircular, on tip of anal tubercle (Fig. 5·C); lateroposterior margin much longer than lateroanterior margin. Apex bluntly pointed, with 2 small and one large stout apical setae on distal part. One large stout discal seta at about apical one-third; 2 to 3 fringe setae on each side.

Anal plate length of each instar is shown in Fig. 1.

GLAND PORES AND DUCTS

Structure

There are six types of dermal gland pores or ducts as follows.

1) *Simple pore* (Fig. 3·B1-5): Consists of one duct with one to 4 loculi at opening as in figures: unilocular (Fig. 3·B1); bilocular, complete (Fig. 3·B3) and incomplete (Fig. 3·B2); trilocular (Fig. 3·B4), and quadrilocular (Fig. 3·B5). Outer wall strongly sclerotized. Variable in size.

Both third- and fourth-instar insects have pores of this type on the dorsum.

2) *Cruciform pore* (Fig. 3·B6): Minute, semicircular, with central cruciform orifice; wall weakly sclerotized.

All instars have pores of this type on the venter.

3) *Quinquelocular disc pore* (Fig. 3·B7): Discal, usually somewhat pentagonal, with 5 radial walls extending from a minute central cell.

All the instars have pores of this type along the stigmatic furrows.

4) *Multilocular disc pore* (Fig. 3·B8): Discal, larger than quinquelocular disc pore, composed of one delicate outer ring with one inner circle of about 10 continuous loculi. Somewhat similar to lemon slice in shape.

5) *Tubular duct* (Fig. 3·B9): Extremely delicate tube, with one sclerotized inner terminal cap, and with one filament on margin of cap, the filament slender and as long as duct itself; orifice of duct simple.

Only the fourth instar has ducts of this type opened on the surface of the venter.

6) *Filamentous duct* (Fig. 3·B10): Excessively small and fine, orifice simple; extremity of inner end variously branched.

Both the third and fourth instars have ducts of this type along the body margin.

Distribution

First instar (Fig. 2·A): A few cruciform pores on submarginal area of venter. Two to 5 quinquelocular disc pores along each stigmatic furrow.

Neither pores nor ducts could be found on the dorsum by the present method.

Second instar (Fig. 3·A): Many cruciform pores on submarginal area and a few on median area of venter. Seven to 10 quinquelocular disc pores along each stigmatic furrow. A considerable number of very minute pores on dorsum.

The type of the minute pores on the dorsum could not be distinctly determined, but they seemed to belong to the unilocular or incomplete bilocular type of a simple pore.

Third instar (Fig. 4·A): Many cruciform pores on whole surface of venter, especially on submarginal area. Twenty-three to 30 quinquelocular disc pores along each stigmatic furrow.

Fig. 2. Whole body of 1st instar, antennae, and anal plates of *Ceroplastes pseudoceriferus*. A. Whole body of 1st instar: left, dorsal side; right, ventral side. *cp*: Cruciform pores, *qdp*: Quinquelocular disc pores. B. Antennae 1: 1st instar, 2: 2nd instar, 3: 3rd instar, 4: 4th instar. C. Anal plates; left, external side; right, internal side. 1: 1st instar, 2: 2nd instar, 3: 3rd instar, 4: 4th instar,

Fig. 3. Whole body of 2nd instar, gland pores, and gland ducts of *C. pseudoceriferus*. A. Whole body of 2nd instar; left, dorsal side; right, ventral side, *sp*: Simple pores, *cp*: Cruciform pores, *qdp*: Quinquelocular disc pores. B. Gland pores and gland ducts; measurements in the 4th instar. 1-5: Simple pores; 1: unilocular, 2: bilocular, incomplete, 3: bilocular, complete, 4: trilocular, 5: quadrilocular, 6: Cruciform pore, 7: Quinquelocular disc pore, 8: Multilocular disc pore, 9: Tubular duct, 10: Filamentous duct.

Fig. 4. Whole body of 3rd instar, anal ring of 4th instar, and distal part of posterior legs of *C. pseudoceriferus*. A. Whole body of 3rd instar; left, dorsal side; right, ventral side. *sp*: Simple pores, *fd*: Filamentous ducts, *cp*: Cruciform pores, *qdp*: Quinquelocular disc pores. B. Anal ring of 4th instar. C. Distal part of posterior legs. 1: 1st instar, 2: 2nd instar, 3: 3rd instar, 4: 4th instar.

Fig. 5. Whole body of young adult (4th instar), region of anterior spiracular cleft, and anal tubercle of full grown adult of *C. pseudoceriferus*. A: Whole body of young adult (4th instar); left, dorsal side; right, ventral side. *sp*: Simple pores. *fd*: Filamentous ducts, *cp*: Cruciform pores, *qdp*: Quinelocular disc pores, *mdp*: Multilocular disc pores, *td*: Tubular ducts. B: Region of the anterior spiracular cleft in ventral view. C: Anal tubercle of full grown adult. *ap*: anal plates.

Fig. 6. View of waxy secretions in *C. pseudoceriferus*. A: Dorsal view of 1st instar at 3 days after hatching; *a-n*, submarginal lobe-like waxy secretions; *o-u*, ring-shaped waxy secretions. B: 7 days after hatching of the same; I-VIII, lobe-like waxy projections; X, median waxy process. C: Dorsal view of 2nd instar. D: Dorsal view of 3rd instar. *pw*, white line of powdery wax. E: Lateral view of 3rd instar; *1dw*, dry wax of 1st instar; *2dw*, dry wax of 2nd instar; *3ww*, wet wax of 3rd instar. F: Dorsal view of 4th instar at maturity. G: Lateral view of immature form of 4th instar. *dw*, dry wax of 1st and 2nd instars; *med.ww*, median wet wax; *mar.ww*, marginal wet wax; *pw*, white lines of powdery wax.

Many simple pores mainly of the bilocular type are scattered on the whole surface of the dorsum except for 13 blank areas where no pores or ducts can be found. Several filamentous ducts are along the body margin.

Fourth instar (Fig. 5·A): Many cruciform pores on whole surface of venter, especially on submarginal area. Many quinquelocular disc pores along stigmatic furrows.

Numerous multilocular disc pores in a thick perivulvar cluster, and in one sparse transverse row on each abdominal segment, a few pores present even as far as level of middle coxae. A large number of simple pores of bi-, tri- and quadrilocular types on dorsal surface except for 13 blank areas as in the third instar. Tubular ducts scattered laterad of vulva and several tubular ducts cephalad of basal antennal segment. Many filamentous ducts along body margin.

WAX SECRETION

First instar (Fig. 6·A, B): After settling on the host, the newly hatched larvae began to secrete white fragil wax from both the median and submarginal areas of the dorsum. This wax is called 'dry wax' hereafter. The median area of the dorsum secreted wax in 7 rings, 3 on the prosoma (Fig. 6·A, *o-q*) and 4 on the postsoma (Fig. 6·A, *r-u*). These two groups of rings were separated from each other at the beginning of the secretion but gradually came in contact to form a ladder-like pattern. The inside area of each ring was also gradually filled with dry wax. Thus, the wax on median area of the dorsum grew upward. On the submarginal area 14 secretory pencils of dry wax could be seen on each side at the beginning (Fig. 6·A, *a-n*). As insects grew, adjacent 2 or 3 pencils combined with each other, resulting in 15 lobe-like projections of dry wax surrounding the body: one on the anterior extremity of the body (Fig. 6·B·I), 3 on each side of the prosoma (Fig. 6·B·II-IV), and 4 on each side of the postsoma (Fig. 6·B·V-VIII). These projections of wax elongated beyond the body margin as insects grew. Except for areas with dry wax, the dorsal surface was covered with an extremely thin film of transparent wax. Each stigmatic furrow appeared as a white line of powdery wax which was different from the dry wax in external appearance. The ventral surface bore no visible secretion except for the stigmatic furrows.

Second instar (Fig. 6·C): The old dry wax secreted during the first instar retained on the dorsal body surface. Dry wax of the similar quality was secreted and deposited beneath the old wax from the same regions as in the first instar. The lobe-like waxy projections elongated, making a stellate form. Otherwise, the secretory pattern is similar to that of the first instar.

Third instar (Fig. 6·D): After the second moult, all over the surface of the dorsum was secreted a new type of wax (referred to as "wet wax" hereafter). On the median dorsal area, the old dry wax secreted in the first and second instars (Fig. 6·B, C·X) was pushed up by the newly secreted wet wax, and thus the waxy covering developed into a conical or horn-like structure, a little directing forwards, the boundary between the dry and wet waxes remained distinct (Fig. 6·E). Areas around the submarginal lobe-like projections of dry wax were also filled up with the wet wax. The marginal secretion grew to be rolled up, bringing the stigmatic secretions onto the submarginal dorsum.

Fourth instar (Fig. 6·F): The pattern of the wet wax secretion during this instar was similar to that of the third. Owing to large amount of wet wax secreted on the dorsum, the waxy covering formed a dome, on top of which a very small cone of the old dry wax retained (Fig. 6·G). Dorsal secretion of wet wax covered most part of the submarginal lobe-like projections of the old dry wax, around which shallow depressions occurred. The posterior half of the venter especially around the vulva, was covered with powdery wax. The amount of the powdery wax increased just before the oviposition.

DISCUSSION

The general morphology of the female of *C. pseudoceriferus* elucidated by the present observations is quite the same as that of *C. ceriferus* described by KUWANA (1923). Thus, the female insect described as *C. ceriferus* by KUWANA (1923) must be the female of *C. pseudoceriferus*. The female of *C. pseudoceriferus* moults three times, and the fourth instar corresponds to the adult stage. The male has not been found during the present observations, though KUWANA (1923) described it. No fundamental difference in external structures was found among instars, but some particular differences were recognized. For instance, the form and size of the antennae, spiracular setae and anal plates are apparently different and these characteristics afford important key for distinguishing the instars. The structure and distribution of the pores and ducts on the body surface are also characteristic for each instar.

The secretory patterns of waxy covering have been briefly described by TAMAKI (1963) on *C. pseudoceriferus* and by KUWANA (1923) on some species of *Ceroplastes*. At the moulting the crumpled exuvium is pushed out from the posterior end of the body, keeping the old waxy covering up on the body of the new instar, and the new waxy covering is produced and accumulated under the old one.

The dry wax secreted by the first and second instar larvae looks like a fragile fibroid mass and it does not contain aqueous substance. On the contrary, the wet wax of the third and fourth instar insects is soft and amorphous like paste and contains a large amount of aqueous substance which has been designated as 'interior honeydew' (TAMAKI and KAWAI, 1966). The interior honeydew contains various kinds of amino acid and carbohydrate (TAMAKI, 1964a, b), and waxy substance in the wet wax of the mature adult consists of fatty acids (normal acids ranging from C_8 to C_{32}), unsaponifiable matter (cyclic or branched chain and C_{26} -straight chain) and resin acids (TAMAKI, 1966). The waxy substance separated from the waxy covering of the first and second instars seemed to be different qualitatively from that of the third and fourth instars (TAMAKI and KAWAI, 1966). Differences of the chemical compositions of the waxy substances separated from the two kinds of waxy covering will be reported elsewhere.

The distribution of the pores and ducts on the body surface is very interesting in relation to the formation of waxy covering. On some regions of the dorsum secreting dry wax, neither pores nor ducts were found by the methods used. However, some glands responsible for secretion of dry wax must be present on these regions. Developing into the third instar, the larvae begin to secrete the

wet wax, and this secretion continues in the fourth instar. The fact coincides with the appearance of numerous simple pores on the dorsal body surface of the third and fourth instars. The simple pore consists of a duct and one to four loculi in a complex structure. This suggests that the waxy and aqueous substances are separately secreted by the simple pores and then mingled on the body surface, resulting the wet wax. The distributions of the quinquelocular disc pores and multilocular disc pores coincide in the areas secreting white powdery wax. The former pores are distributed on the stigmatic furrows and the latter on the posterior half of the venter especially around the vulva. These particular pores are commonly found in the family Coccidae; they are considered to secrete the white powdery wax. It could be imagined that the white powdery wax is responsible for the respiration of the insects.

Several tubular ducts were found on the ventral surface of the fourth instar, but their function remained obscure. In some other coccid species belonging to several genera, such as *Pulvinaria* TARGIONI, *Protopulvinaria* COCKERELL, *Takahashia* COCKERELL, and *Metacaronema* TAKAHASHI, the tubular duct seems to participate in the secretion of wooly wax which forms ovisac, while the species of some other genera, such as *Saissetia* DEPLANCHE, *Parasaissetia* TAKAHASHI and *Lecanium* ILLIGER, do not display any obvious waxy secretion in spite of the presence of numerous tubular ducts (KAWAI, unpublished observations). The filamentous ducts are confined in the marginal area of the third and fourth instar insects, but their role is not clear.

In the first and second instar larvae, an extremely thin film of transparent wax is found on the dorsum except for areas covered with dry wax. It is possible that the ventral body surface is coated with similar wax. No gland responsible for the waxy film of the dorsal surface was found during the first instar, but many minute pores were observed in the second instar. Although the type of these minute pores was not determined exactly, they seemed to belong to the type of the simple pores. Thus the thin film of transparent wax on the dorsum is probably similar to the wet wax in quality. The cruciform pore on the ventral surface may be regarded as the gland forming the thin waxy film. It should be mentioned that the waxy secretion of the venter has a role to anchor the insect on host plant. The wet wax secreted from the dorsum beyond the body margin plays the same role during the third and fourth instars. When the adult matures sexually, the venter of the body become separable from the host to make a space for oviposition. Thus the insect in this period seems to settle on the host plant only by the wet wax.

The distributions and structures of the gland pores and ducts were found to be actually correlated with the production and characteristics of the waxy covering of *C. pseudoceriferus*. FERRIS(1950), working on mealy bugs(*Pseudococcidae*), noticed some correlations between the structure of glands and the characteristics of secretions; but he also noticed that the characteristics of secretions often much depended upon physiological conditions of these insects rather than upon the structural differentiations of the gland pores and ducts. As to the pores and ducts of the scale insects much has been done from the taxonomical point of view, but unfortunately there are few reports on the relations between these glands and any waxy covering. Further detailed studies on various scale insects

are necessary in order to get sound information on the function of gland pores and ducts. It should be stressed that the adoption of histochemical techniques is essential and quite promising in advancing this problem.

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