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## Morphology, systematics and genetics

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### Changes in body size and shape in *Aonidiella aurantii* (Maskell 1879) adult female

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**Abstract:** Adult body size and shape changes during female adult (reproductive) life is a common phenomenon in Coccoidea. Moreover, some Diaspididae species show remarkable sclerotization of the whole body rather than just part of it in mature adult females.

This study clarifies the way in which the body size and shape changes, plus how the sclerotization takes place. These changes could be the result of a simple cuticular stretching, or an epithelial hypertrophy, or an epithelial hyperplasia combined with a sclerotization taking place over a defined short period or during the whole shape and size change process.

Live adult females of *Aonidiella aurantii* were chosen as subjects because of their typical growth pattern during adult instar. Adult females from teneral to fully grown and mature older specimens were stained with DAPI, a fluorescent nuclear stain and observed under a Zeiss Photomicroscope III, equipped with a HBO Mercury Short Arc Lamp. The evidence shows that the changes in body size and shape and the sclerotization of the prosoma of *A. aurantii* adult females are coordinated phenomena originating from the hypertrophy of the epithelial cell layer. Moreover, the so called “bosses” appear to be the first signs of sclerotization.

**Key words:** functional morphology, post-embryonic development.

### Introduction

The occurrence of changes in size and shape of adult females of Diaspididae is well-known. Takagi (1990) reported that the adult females “greatly increase in size after their emergence” and “the cuticle expands by the pulling out of wrinkles which are densely crowded over the body in the teneral adult female”. Takagi (1990) wrote that: “The teneral and full-grown female may differ greatly, owing to differential growth of a particular part of the body”.

The Diaspididae, and perhaps all of the Coccoidea as well, grow in size and change their shape during adult life unlike most other orders of insects.

So the subject of this study is to give a contribution about the way this unusual behaviour takes place in adult females of Diaspididae.

The choice of *Aonidiella aurantii* as the subject of investigation was to focus the study on its prosoma. The anterior part of the body of *A. aurantii* undergoes clear changes during adult life but it doesn't have cuticular wrinkles or folding. This should make it possible to give a better interpretation of the observed phenomena, taking into account “the relation between structure and function” (Takagi, 1990).

## **Material and methods**

Specimens of *A. aurantii* were collected from on a large ornamental Rose (*Rosa* sp.) and *Citrus aurantium* L. plants growing in the Agriculture Faculty garden. *A. aurantii* specimens on the collected twigs and branchlets were immediately processed as follows.

Live adult females of different ages (teneral, fully grown and aged) were selected and then dipped into a watch glass with cold methanol on ice. Each insect was immediately dissected to obtain good fixation and staining by means of a large opening in the cuticle. Soon each specimen was moved into an Eppendorf vial filled with fresh methanol and sunk into ice where they were rested for about 5 minutes. Methanol was substituted by acetone for 1 minute and then the specimens were washed for 20 minutes in the dark by PBS (1X = 0.8 gr NaCl + 0.2 gr KCl + 1.15 gr Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O + 0.2 gr KH<sub>2</sub>PO<sub>4</sub> + H<sub>2</sub>O up to 1 l) + 0.5% acetic acid + 1% Triton X-100.

The specimens were stained for 15 minutes in DAPI (4',6-Diamidino-2-phenylindole dihydrochloride): 5 µg dissolved into 10 ml SSC (0.3 M sodium citrate + 3 M NaCl: pH ~7.0) buffer.

After the staining, the insects were moved back to PBS (1X) and washed for 15 minutes, then transferred into a PVA anti-fading mounting medium with Dabco™ by Fluka BioChemika where they rested in the dark for at least three days in order to counterstain. The adult females were thick mounted with a coverslip in the same anti-fade PVA. Thick slide mount was preferred in order to preserve the spatial architecture of the tissue and cuticle.

Observations were performed by transmitted light with a Zeiss Photomicroscope III. The microscope was equipped both with a 100W bulb lamp and a 50W Mercury Short Arc Lamp (Osram HBO 50 W/AC). Pictures were taken with an Olympus E330 digital camera. An appropriate substage condenser for bright and dark field was used. UV bandpass filter was added on the field diaphragm of the microscope in order to cut off intense visible light produced by the HBO lamp.

Observations on body size were performed in bright field on teneral and adult females slide-mounted in Canada balsam as suggested by Wilkey (1990). Additional observations were obtained on prosomal transverse sections of fully grown adult females. The specimens were cleared as suggested but the 20-40 µm sections were mounted in Hoyer's medium in such a way as to expose the cut surface. The salivary pump was chosen as the body centre in order to compare the relative size of the different regions.

## **Results**

### *Fluorescence*

DAPI stained adult females from teneral to fully-grown show green fluorescence when observed under UV light. The fluorescence arises from numerous small, clear green, almost spherical structures. These structures are here recognized as cell nuclei on the basis of the selective behaviour of DAPI that stains DNA. Up to 5 % of the nuclei, appeared stretched, and were between five/ten times longer than wide.

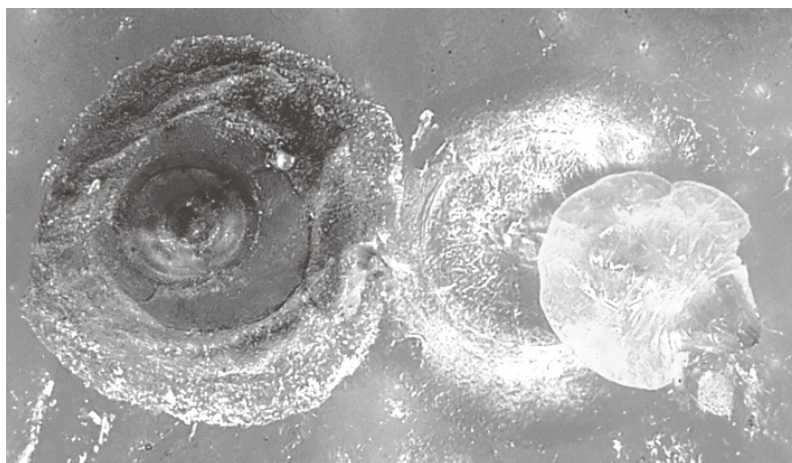
No condensed chromosomes were observed but some rare and uncertain structures were observed in the place of the body possibly occupied by ovaries. These structures can be compared with those shown by Porcelli *et al.* (2005).

In teneral or young adult females (Fig. 1) the fluorescence of the whole body is not uniform (Fig. 2a). A central very fluorescent area surrounds the mouthparts and fills the abdomen and the pygidium: this region is so rich in nuclei that it is not easily resolved in the thick slide mounts here presented. On the other hand, some nuclei could be resolved even here, by focusing. In contrast, the peripheral area of the prosoma shows clear nuclei. In the teneral adult female this area is equal to 1/4 of the prosomal diameter and runs from one lateral lobe to the other.

Studying a series of teneral and differently aged specimens, it is evident that in each growing female the lacunae are larger on the dorsum in comparison to the inter-antennal area, smaller on both sides of the prosoma and absent at the apex of the lobes (Fig. 2b). As a consequence of the lacunae, the nuclei are absent or displaced and their density is lower in the dorsal inter-antennal area but their density increases up to the apex of the lobes (Fig. 2c). During aging, the marginal area of the prosoma increases by “differential growth” and attains more than 1/3 of the prosomal diameter.

In the fully-grown female (Fig. 3a) the lacunae are larger and more uniformly distributed than in teneral adults. The nuclear overcrowding is still perceivable at the prosomal margin, and it seems that the nuclei form now a single cellular layer.

Once the female has attained its final size, sclerotization of the prosoma and cuticular bosses appears (Ben-Dov, 1990; Takagi, 1990) (Fig. 3c). Studying the same specimen by UV fluorescence (Fig. 3a) and bright field (Fig. 3c) one can observe that there are no nuclei associated with each lacuna.

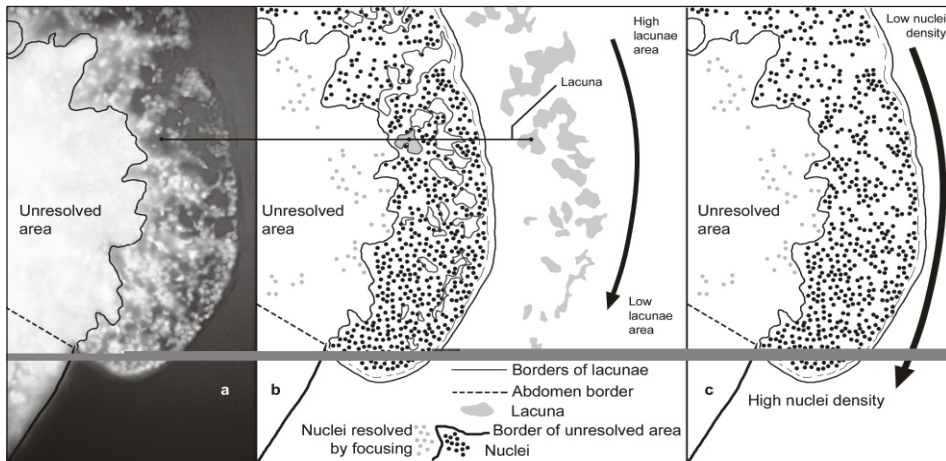


**Figure 1.** *Aonidiella aurantii*: this teneral adult female is just starting to develop its lobe. This is the best time to perform observations by fluorescence.

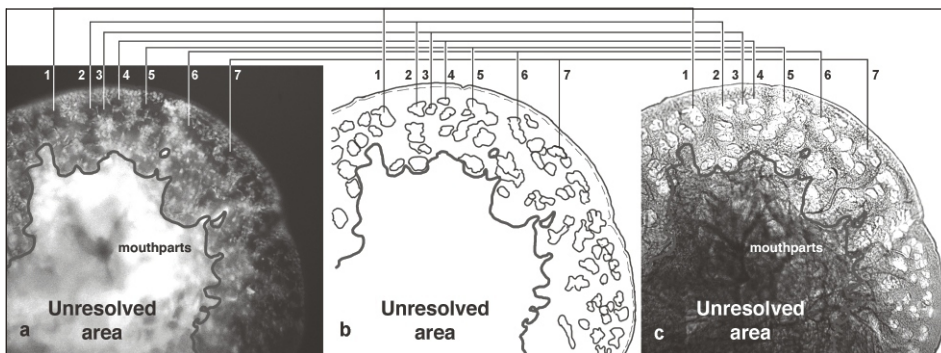
Aged females do not show any fluorescence in the peripheral area of the prosoma but do show it in the unresolved area, possibly due to the tissues of the reproductive or the digestive apparatus.

## Body size

In the teneral adult female (Fig. 4) the body is elongate and the salivary pump is placed at the first third of the total body length. The distance from the anterior prosomal border and the lobe apex is comparable with the body span at the salivary pump level. The distance between the right spiracle and the respective prosomal border is something less than two and a half times the width of mouthparts. Segments of the abdomen and the pygidium are prominent. The entire cuticle is membranous and cuticular folds or wrinkles are absent. Teneral females average 0.8 mm long and 0.6 mm wide.



**Figure 2.** (a) Right side of a young adult female as in Fig.1 stained with DAPI and observed in fluorescence; (b) schematic drawing to show the distribution and the area of lacunae; (c) schematic drawing to show the density of nuclei. The arrows show the sense of clines in area of lacunae (b) and nuclei density (c).



**Figure 3.** Cephalic and right part of the prosoma of *Aonidiella aurantii* at the time of sclerotization: (a) as appears in fluorescence; (b) schematic drawings to show correspondence between lacunae in (a) and bosses in the bright field image in (c). This last image was obtained putting offline the UV passband filter and closing both field and aperture diaphragms.

The full growth female (Fig. 4) shows considerable thickening and sclerotization of the cuticle on the whole prosoma. Few (two or three) folds are visible as sclerotized ridges between the prosoma and the abdomen. The salivary pump is located at a little less than half of the body length. The body span is comparable with the distance between the anterior prosomal margin and the apex of the opposite lobe (Fig. 4). Full growth individuals are about 1.1 mm long and 1.3 mm wide. In full growth females the distance between the right spiracle and the respective prosomal border is about five times the width of mouthparts.

Finally the cuticle of the prosoma of an aged adult female in transverse section is considerably thicker at the dorsum than at the venter. Moreover the dorsal cuticle is much more sclerotized than the ventral cuticle (Fig. 5).

## Discussion

### *Nuclei and cells*

The DAPI stains consistently the nuclei of *A. aurantii* and the unresolved area could result from both a large amount of nuclei and some inadequacy in counterstaining. The body centre of *A. aurantii* hosts several important systems: reproductive, digestive and nervous, so over staining is expected there.

In the peripheral area of prosoma the nuclei are clearly resolved and no signs of mitoses are present. Some mitoses could be present, of course, but the low number would be too insignificant to account for the prosomal growth. So the hyperplasia prosomal growth hypothesis is here discarded.

The vertical distribution of nuclei is here interpreted with the occurrence of two epithelia: a dorsal one, that is rich in nuclei, and a ventral one made up of many fewer cells. The observed figure is the result of a two layer superimposition.

### *Prosoma*

The prosomal swelling starts in teneral (just moulted) females with incoming lacunae in the "frontal region" then lacunae enlarge and new ones appear towards the apices of lobes.

Once present, the lacunae enlarge, attaining more or less the same surface as the swollen prosoma and reach the final size. The process of prosomal growth acts like a wave: starting from the frontal region and ending at the apices of lobes.

Once grown the prosomal cuticular thickening starts with the occurrence of first bosses. In the figure (3c) the bosses appear clear on grey background. This unusual aspect depends on the fact that no tissue underlines each boss, while cells and tissues are seen as granulose matter around as a consequence of the very narrow field and aperture diaphragm stops.

A clear relationship between lacunae and bosses do exist. Actually one can observe a lacuna just behind each boss (Fig. 3). The bosses are here interpreted as the place of first thickening of the dorsal cuticle. As a consequence of the correspondence between lacunae and bosses it is here inferred that the lacunae could be present in the dorsal epithelium.

On further growth, the bosses connect with each other, the nuclei and perhaps the cells are lost, and the prosomal dorsal cuticle thickening is completed.

It is maintained that in *A. aurantii* neither cuticular stretching nor "pulling out of wrinkles" are observed but a real growth.

In favour of this interpretation is the fact that the gain in length of fully growth female is due to the increased distance between salivary pump and antennae (Fig. 4: body length)

that becomes two times longer than in the teneral female. A further important dimensional gain occurs in full growth female span that is about three times larger than in the teneral one. This gain is mainly due to the cuticular growth between the spiracle and the lateral prosomal border. Each side enlarges two times (Fig. 4c1-c2) so that the total gain of the body span is two times compared to the teneral female.

For the above reasons the inference that prosomal growth depends mainly by hypertrophy and production of new cuticle where lacunae and bosses are present, is here accepted. These processes end with the prosomal sclerotization.

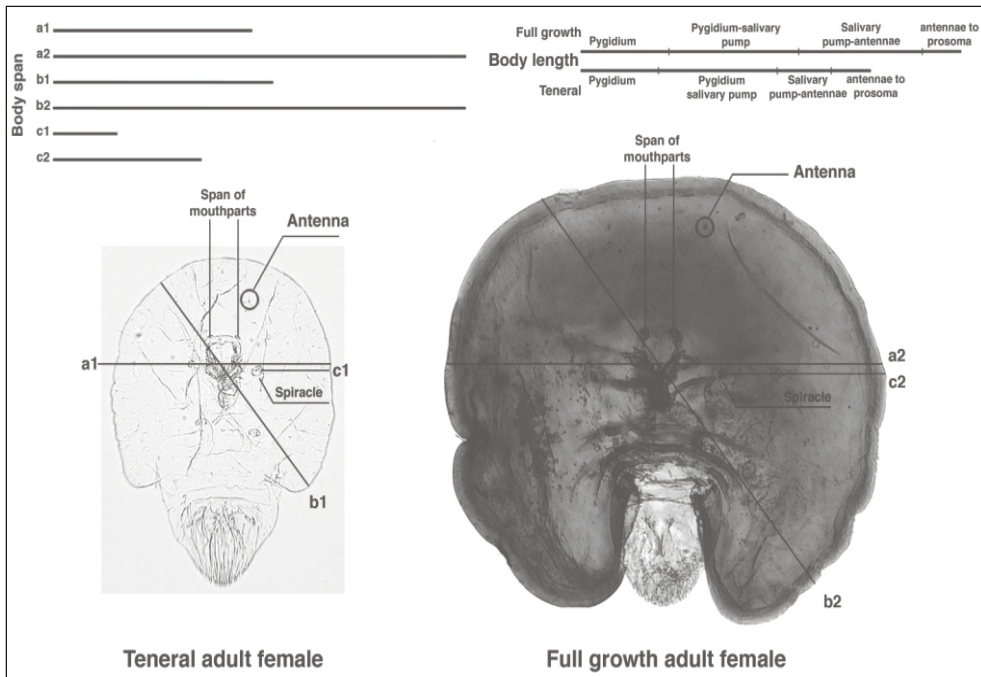
### Functional morphology

The possible function of this particular mode of development and morphology of the adult female could be to harbour the largest possible number of eggs. It could be reasonable to infer that a larger prosoma has room enough for a larger reproductive system in *A. aurantii*.

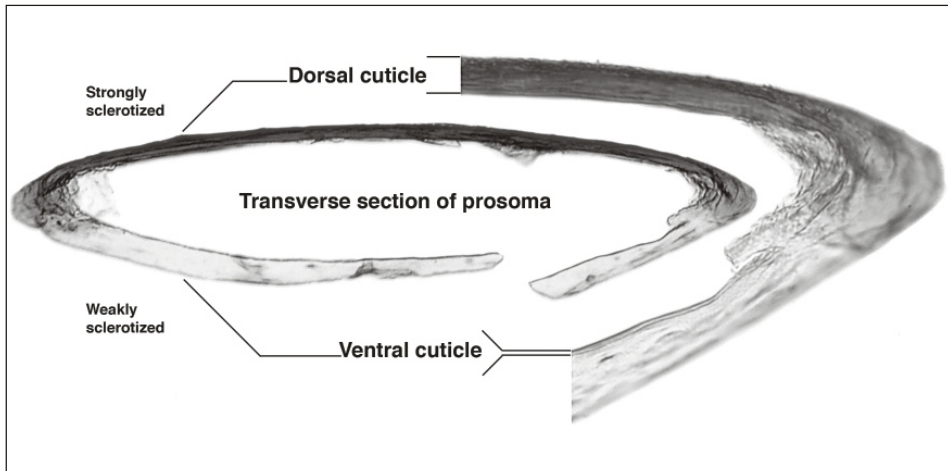
But there is no need to evolve this really unusual mechanism for this purpose, because the second instar could directly give rise to a larger adult female, whether capable of sclerotization of its cuticle or not.

So the interpretation could be that growth during the adult instar, as *A. aurantii* does, makes it possible to reach a better reproductive fitness by optimising the use of food and time in relation to the host plant or the climate.

Lastly, although the depicted process looks like physogastry as its function, but occurs actually on the prosoma.



**Figure 4.** *Aonidiella aurantii*: comparison between relative body measures of a teneral (in phase contrast) and a fully growth (in bright field) adult female.



**Figure 5.** Transverse section of prosoma of an aged female of *Aonidiella aurantii*. Dorsal cuticle is evidently much more sclerotized than the ventral cuticle.

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