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Cellular Processes in the Growth of Lithobiomorph Centipedes (Chilopoda: Lithobiomorpha). A Cuticular View

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Abstract. The cuticle of lithobiomorph centipedes (Chilopoda, Lithobiomorpha) offers a special opportunity for studying cellular processes of morphogenesis during postembryonic growth.
The present paper shows that in lithobiomorph centipedes the polygonal surface pattern of the cuticle can record the geometry of the external face of hypodermal cells at the stage of deposition of the very first layers of the cuticle (epicuticle).
Based on this hypodermis-to-cuticle correspondence, cuticular patterns are used to study the hypodermal behaviour during growth of an area of the cephalic shield. Growth is isometric and intercalary and mitosis is the fundamental cellular process responsible for its realization, but adjustments of cell size and shape are also extensively involved in the global control of sclerite form. The observed spatial distribution of mitoses is evaluated against the statistics predicted by a null model of random distribution. The observed growth patterns show a character of local randomness, but some constraints at the level of the whole sclerite seem to be at work. No effect of lateral inhibition is observed.

Key words. Integument, cuticular patterns, scutes, postembryonic growth, morphogenesis, mitosis.

1. INTRODUCTION

Morphogenesis is the outcome of a limited repertoire of cellular processes: 1) cell division; 2) changes in cell shape; 3) cell movement; 4) cell growth; 5) cell death; 6) changes in the composition of cell membrane and extracellular matrix (GILBERT 1997), but our understanding of the relationship between these basic cellular processes and the acquisition of shape and form during ontogeny is not well advanced. Although molecular genetics is providing a clearer picture of cell differentiation, we still have a very imprecise notion of the quantitative control of cell proliferation that leads to a specific size and shape (GARCIA BELLIDO et al. 1994).
In arthropods, cellular events occurring during postembryonic development in the epidermis (hypodermis) are often recorded by changes in number and arrangement of normal and specialized setae, or in the sculpture of the cuticle, but this opportunity has seldom been exploited, except for the evidence provided by the spatial distribution of setae on the surface of *Drosophila* wings (e.g. GUBB 1998) and scales on butterfly wings (e.g. NIJHOUT 1991; MONTEIRO et al. 1997). In this paper we show how much information on hypodermal cell events can be obtained from the comparison of cuticular patterns in subsequent developmental stages of the same individual, in those (widespread) cases where the cuticle shows a detailed "frozen blueprint" of the hypodermal cells at the time of cuticle secretion. We will demonstrate this possibility using the cephalic shield of lithobiomorph centipedes (Lithobiomorpha, Chilopoda).
Centipede integument ultrastructure has been reviewed by LEWIS (1981) and, more recently, by MINELLI (1993). Centipedes' hypodermis rests proximally on a basement membrane and is covered externally by a cuticle. In general, the sclerites' cuticle consists of three main layers: 1) the external epicuticle, highly refractile, up to 1 µm thick; 2) the exocuticle, rigid and generally amber coloured; 3) the lamellated endocuticle, thick, elastic and colourless. In regions of transition between neighbouring sclerites (the so called arthrodial membranes), in proximity to sensory organs and in the cavities opening at the body surface (tracheae, fore- and hindgut), the structure of the cuticle may be quite different (BLOWER 1951). In *Lithobius forficatus* (Lithobiomorpha) the sclerite cuticle may be up to 50 µm

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thick with a 5–6 µm exocuticle and an epicuticle about 1 µm thick (Füller 1963).

In surface view, the cuticle of centipedes often displays a polygonal pattern (Fig. 1). The individual polygonal fields (*scutes* sensu Cals 1974) forming this pattern are generally regarded as corresponding to the outer faces of the hypodermal cells, but this has never been directly shown in centipedes (Minelli 1993). A more or less precise correspondence between cuticular patterns and the geometry of lateral cell-to-cell contacts in the hypodermis is often assumed in other arthropods as well (see Grassé 1975 for insects). The unicellular origin of polygonal scutes (hereafter: scutes) has been suggested for insects (see for instance Hinton 1970; Cals 1973) and for crustaceans (see for instance Cals 1973; Cheng & Chang 1993) generally on the basis of the agreement between the scute density and the density of cell nuclei in the hypodermis.

In insects, although the relationship between the geometry of cuticular sculpture and the arrangement of hypodermal cells has not been directly investigated, the production of new cuticle at each moulting cycle and the relationship between its structure and the arrangement and activity of hypodermal cells have been studied in depth in a series of classic papers (up to date reviews in Locke 1998 and Neville 1998).

Locke (1966, 1969) investigated the origin of cuticular surface patterns in the caterpillar *Calpodes ethlius* (Lepidoptera: Hesperiidae) during the moult from fourth to fifth instar larva. In particular, the cuticular patterns around the wax-secreting glands show polygons with raised edges, that is the opposite of the scutes in centipedes, which have sunken edges. Cuticulin (external epicuticle), 10–20 nm thick, is the first layer deposited when the new cuticle is formed. It grows by accretion at the edges of secreted patches of cuticulin which increase in area until the whole cell's surface is covered. Locke's observations support the hypothesis that various cuticular patterns arise by buckling of the cuticulin layer as it increases in surface area. Accordingly, differences in pattern are attributable to: a) the shape of the surface when the cuticulin is secreted; b) the distribution (both spatial and temporal) and the differential rate of deposition of cuticulin over this sur-

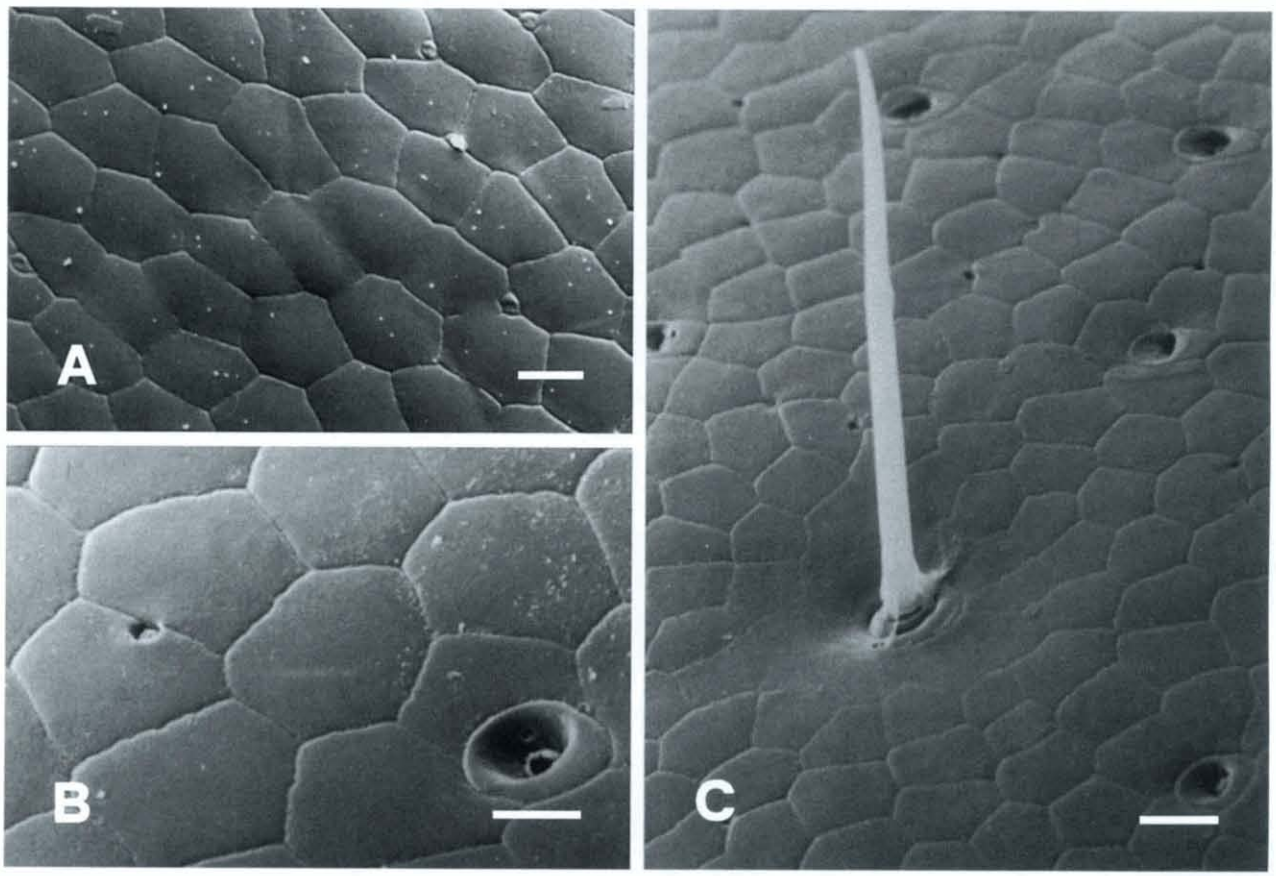

Fig. 1. Surface view of centipede cuticle (SEM microscopy). Polygonal pattern and some specialized structures: setae, pore cells, micropores. **A**: *Clinopodes flavidus* C.L. Koch, 1847 (Geophilomorpha), forcipular coxosternum, bar = 5 µm; **B**: *Eupolybothrus grossipes* (C.L. Koch, 1847) (Lithobiomorpha), cephalic shield, bar = 5 µm; **C**: *E. grossipes*, cephalic shield, bar = 10 µm.

face; c) the stresses and subsequent buckling resulting from a) and b). In LOCKE's model the shape of the hypodermis is driven by the physical forces involved in the building of the cuticulin layer. The geometry of hypodermis only works as "morphological seed" for setting up a process of accretion that barely records the spatial configuration of the cells.

WIGGLESWORTH (1973) studied the development of cuticular patterns, like stellate foldings and transverse ripples, in the bug *Rhodnius* (Heteroptera: Reduviidae). In this material, polygonal patterns, or patterns with different shape, whose individual elements nevertheless are likewise derived from one cell each, appear only transiently during cuticle shaping. For instance, the formation of the stellate cuticular pattern of the 5th-stage larval abdomen begins with a first phase when the cuticle presents a honeycomb-like pattern with folds raised over lateral cell boundaries; later the epicuticle folds in the shape of little stars (one per cell) which finally fuse into groups of 2–5 units to form large composite stars without precise correspondence to the geometry of hypodermis. During the formation of the cuticle in the adult abdomen, where the hypodermal cells are transversely elongated, the transverse ripple pattern of the adult develops by a similar process: the elongated stars tend to fuse into transverse chains.

Our study demonstrates (see below, Results) that in Chilopoda the main process of cuticle formation results in simple *coating* of the hypodermis, not involving any special mechanical force attributable to the lateral growth of the epicuticle. Cuticle surface can thus reproduce the shape of the external side of the hypodermis exactly. This is the case for a large part of body surface in centipedes and probably also for localized regions of the cuticle in many other arthropods.

A possible precise correspondence between cuticular polygonal pattern (CuP) and the pattern of the outer face of the hypodermis (CeP) is not easy to test. CuP is observable and stabilized after ecdysis (the emergence from the exuvia), while the CeP we want to compare with it occurs transiently just after the apolysis (the freeing of the hypodermal cells from the old exoskeleton). In lithobiomorphs the latter event takes place a few days before the ecdysis (JOLY 1966). We face the same problem as when testing for correspondence between a cast and its presumptive mark when these are not accessible at the same time. We will consider as an indirect but reliable proof of the correspondence between CuP and CeP the observed high degree of local conservation of CuP across developmental stages, a conservation that requires a templating process of cuticle shaping. We will show that, under certain conditions, scute configuration is stable across subsequent developmental stages, entailing processes of cuticle deposition and shaping that are highly repeatable during post-embryonic development, at the resolution of individual scutes. The only cuticle-shaping mechanism able to guarantee the level of repeatability we recorded seems to be the coating of a permanent structure (a *template*, i.e. the outer face of hypodermis), allowing for the reproduction of its surface geometry. If the CuP were only the result of the interplay between cell secreting activity, structural stress and strain due to lateral accretion of cuticulin patches and local physical and chemical conditions, we would expect no more than statistical similarity between two subsequent exuviae of the same individual, e.g. in scute average size and orientation, but this is not what we see.

2. MATERIALS AND METHODS

2.1. Materials

This study is mainly based on specimens of *Lithobius muticus* C. L. Koch, 1847 and *L. agilis* C.L. Koch, 1847 collected in a small lowland woodland (Bosco di Carpenedo, 10 km NW of Venice, Italy) in June–July 1997. The centipedes were kept in individual containers, fed *ad libitum* with living adult *Drosophila* and checked for moulting almost daily. Additional specimens of *L. muticus*, used for transmission electron microscopy, were collected in the same locality in May 1998.

2.2. DAPI staining

To estimate the density of hypodermal cells, 4 specimens of *L. muticus* and one specimen of *L. agilis* were fixed in 70% ethanol, then incubated for 24 h at 4 °C in DAPI (Diamidino-Phenylindole) solution at the concentration of 40 µg/ml PBS (Phosphate Buffered Saline, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4). Stained specimens were observed with a fluorescence microscope (Zeiss Axioskop) under UV light.

2.3. Cuticle ultrastructure

Among lithobiomorphs, data for *L. forficatus* only are available in the literature (reviewed in MINELLI 1993), therefore integument ultrastructure was studied on 5 specimens of *L. muticus* fixed at different intermoult phases. The body was transversely cut into pieces 1–2 mm in length which were immediately fixed in 2% glutaraldehyde (0.1 M cacodylate buffer, pH = 7.3) for 3 h in ice, then repeatedly washed in the buffer, postfixed with 1% OsO_4 in the same buffer for 1 h, dehydrated in ethanol, and embedded in Epon. Ultrathin sections were counterstained with uranyl acetate and lead citrate and observed with an electron microscope (Hitachi H 600).

2.4. Cuticle preparations

Exuviae were collected when left by moulting animals, otherwise exuvia-like preparations were obtained after fixation of whole specimens in 70% ethanol followed by proteolytic digestion, modified from HILKEN's (1994) protocol. The speci-

mens were incubated in 50 mM $(NH_4)_2CO_3$ adjusted to pH 8 with Na_2CO_3 for 24 h at 37 °C, then digested in trypsin (1 mg/ml) in the same solution at 37 °C for 2 to 7 d, depending on body size. After complete digestion, the remaining cuticle was washed in water and stored in 70% ethanol. Selected sclerites of the exuviae and exuvia-like preparations were mounted on ordinary glass slides with glycerol.

2.5. Morphometric analysis

In order to compare CuPs between subsequent developmental stages of the same individual, 36 cephalic shields from 18 pairs of exuviae of contiguous stages belonging to 18 specimens of *L. muticus* of different ages (from II larval to adult stage) were photographed through a phase contrast microscope (Zeiss Axioskope). The film frames were subsequently digitalized using a scanner (Polaroid SprintScan 35) at a resolution of 1012 × 1012 dpi, then submitted to computerized morphometric analysis.

Four landmarks at the edges of the cephalic shield were chosen for estimating the developmental stage and for calculating the individual linear growth rate and the allometric growth pattern of the sclerite. For allometry analysis, five more *L. muticus* specimens were added to the sample.

The following analyses, involving measures of scutes or the position of cuticular structures, are focused on a restricted area of the median-anterior zone of the cephalic shield, a quadrilateral area identified by four idionymic setae, i.e. setae individually distinguishable from the others and recognizable as homologous in different specimens (in this case, also in different species). In adult specimens, this area comprises some 10^3 scutes, whereas those on the whole cephalic shield are in the order of 10^4. As in the remainder of the cephalic shield, specialized cuticular structures (*organules* sensu LAWRENCE 1966) like setae and pores are clearly recognizable in the selected area. At moulting, the organules of the previous stage are mostly retained. Despite the first occurrence of some new setae and pores, their relative position is also quite stable (cfr. KEIL 1975, 1997 for sensilla in *Lithobius* and in arthropods generally). Nevertheless, in order to compare homologous areas of the cuticle from different developmental stages of the same individual, a preliminary test of the reliability of the spatial configuration of the organules was performed. In 4 specimens of *L. muticus*, 30-50 landmark/organules were chosen in the median-anterior zone of the cephalic shield and compared within pairs of exuviae representing contiguous stages of the same specimen. The configurations of landmarks in the two stages were compared by processing the digitalized picture with the software package TPS developed by F.J. Rohlf at the State University of New York at Stony Brook. Generalized least-squares superimposition analysis (GLS, ROHLF & SLICE 1990) allows the comparison of different configurations of landmarks in a geometric space of pure shape, i.e. after removing the effects of size, and misplacements due to translation and rotation.

For each specimen, 2–5 small areas of the cuticle of the younger stage (some tens of scutes each) were sampled from the median-anterior zone of the cephalic shield for calculating the scute average size (area) and the average density of organules.

For each specimen, small areas of the cuticle, delimited by recognizable landmarks, were sampled from the median-anterior zone of the cephalic shield for comparing the geometry of the cuticular pattern in the two stages of the same individual.

Three specimens were chosen for studying the spatial distribution of mitoses. Using pore cells as landmarks, 122 areas with an approximate size between 11 and 225 scutes (cells) were sampled and their quadratic growth rate measured as the ratio between the areas in the two stages. For each specimen the sampled areas were grouped according to size (2–3 classes for each specimen) and the relative variations (Pearson's Coefficient of Variation, C.V.) of the quadratic growth rates for different size classes were calculated. The C.V. values calculated from the observed data were compared to the values predicted by a null model of random distribution of mitoses (see Appendix).

3. RESULTS

3.1. Hypodermis-to-cuticle correspondence

3.1.1. Numerical correspondence between cell nuclei in the hypodermis and scutes. By counting polygonal fields on the cuticle and DAPI-stained nuclei in hypodermis in small sample areas of the integument, we found that these elements occur at the same density, that is, scutes and the outer faces of the hypodermal cells have the same average size. This supports a cellular origin of the pattern, as remarked by HINTON (1970) for insects, but does not guarantee that CuP corresponds geometrically to CeP (see below).

3.1.2. Cuticle ultrastructure. Thin sections of *L. muticus* cuticle confirm the general structure described by BLOWER (1951) and FÜLLER (1963, 1965) for *L. forficatus*, despite the conspicuously thinner cuticle (on average, 6–7 µm in *L. muticus* vs. 20–30 µm in *L. forficatus*) (Fig. 2A). In an adult male of *L. muticus*, on the top of the tergite the epicuticle is 0.1 µm, the exocuticle 0.8 µm and the endocuticle 5–6 µm thick. The grooves corresponding to the sunken edges of the scutes are only about 0.1 µm deep. A thin external layer of epicuticle (10–20 nm) follows the outline of the furrows while a thicker internal layer of epicuticle is engraved. The lamellate structure of exocuticle and endocuticle show no disturbance in relation to the outer sculpture. Therefore the polygonal surface pattern is frozen at a stage corresponding to the very first phase of cuticle deposition thus mirroring the geometry of the external face of hypodermal cells at the same stage (see below).

In one specimen fixed 3 or 4 days after moulting, a fine-grained sculpture is visible on the surface of the cuticle (Fig. 2B). The cuticulin is clustered in small domed patches, 0.2–0.3 µm in diameter, similar to those observed in insects at the very first stage of cuticulin deposition (LOCKE 1966). But at the stage this

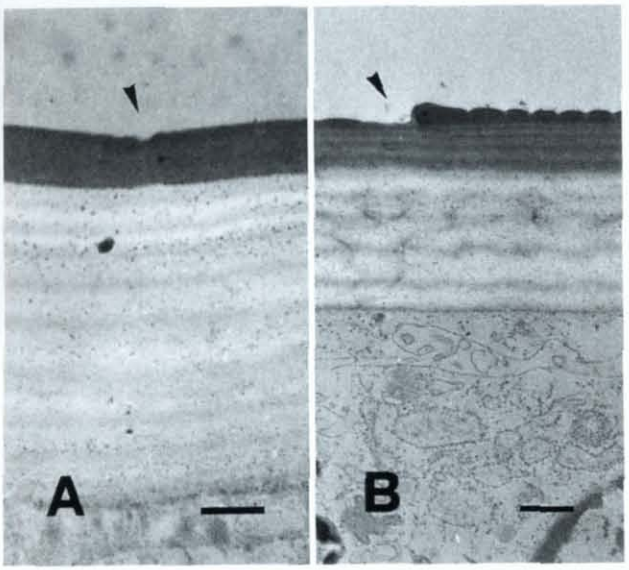

Fig. 2. *Transverse section of the integument of Lithobius muticus* (TEM microscopy). **A**: Fully developed cuticle, the main layers and a furrow between two polygonal fields (arrow heads) is visible; bar = 1 µm; **B**: Cuticle 3-4 days after ecdysis. The 'step' (arrow heads) is the lateral border of a cuticular scale. Small domed cuticulin patches are visible (see text); bar = 0.5 µm.

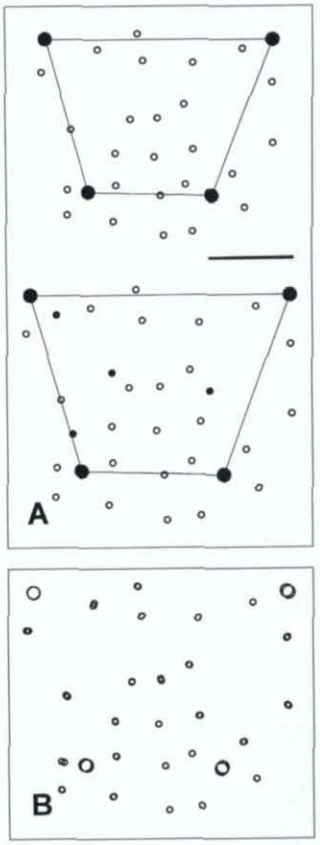

Fig. 3. Schematic representations of a small area of the cephalic shield in two subsequent developmental stages (III to IV postlarva, growth rate = 1.14) of the same individual of *Lithobius muticus*. The size of symbols is approximately to scale. **A:** Landmark configurations at the same scale; large dark circles, idionymic setae; small open circles, pore cells; small dark circles, new pore cells. Lines are for visual aid only, bar = 0.1 mm. **B:** The same two landmark configurations superimposed after isometric scaling to the same centroid size (*GLS procedure*).

specimen was fixed, the exocuticle and even the first layers of endocuticle had been deposited too and the ecdysis was long since completed. We do not know the reasons for the occasional conservation of cuticulin patches in a later stage of cuticle deposition, but this occurrence suggests a mechanism of cuticle deposition qualitatively comparable to that observed in insects and other arthropods (cf. ZIEGLER 1997 for isopods), although perhaps under a different time schedule.

3.1.3. Identification of homologous areas across stages; reliability of landmarks. After isometric scaling to the same centroid size, translation and rotation that minimize square differences (GLS fitting), the geometric configuration of landmarks (both pores and setae but not micropores) of the older stage matches quite precisely the configuration of the same organules in the immediately previous stage. The four specimens tested all had an almost normal growth for their stage (linear growth rate = 1.09–1.14). In the more advanced stage, new organules were added to those already existing, but without detectable alteration of the overall configuration, as in the example in Fig. 3.

In sampled areas of the median-anterior zone of the cephalic shield the average density of organules is 188/mm^2 (n = 18), but the variability among specimens is conspicuous (s.d. = 68, n = 18). We found no significant correlation between the average density of organules and the developmental stage (r = 0.18, n = 18).

Distance between neighbouring organules is in the order of a few tens of micrometers and thus provides a fine marking of the cuticular surface that allows identification of a meshwork of small areas of scutes and comparisons of the patterns expressed by the same animal at different developmental stages (see below).

3.1.4. Conservation of CuP across stages. As anticipated in the Introduction, demonstration of correspondence is based on the conservation of CuP across stages. The degree of correspondence between CuPs of successive exuviae decreases quickly with increasing inter-

moult metric growth. The more growth, i.e. the larger the number of mitoses and the accompanying spatial adjustment of cells, the larger the variation in the geometry of cell-to-cell lateral contacts. The conservation of the pattern is more conspicuous in the cases of moulting with almost no metric growth, that is, with low average density of mitotic events per unit area.

Figure 4 illustrates the highest level of conservation of CuP we recorded. For this specimen and moult, linear growth was almost zero (growth rate = 1.01). The expected low density of cellular events allows for the occurrence of unchanged areas of some 20–30 scutes. All scutes in the two selected areas are individually recognizable in both stages. Modest shape variation is observed in a few scutes, but the topology of lateral contacts is highly conserved. In other areas of the same specimen and in other specimens, conservation is limited to smaller groups of scutes separated by zones of configurational change. The size of the "islands" of unchanged scutes is inversely related to growth rate.

The only plausible mechanism of cuticle shaping able to account for this level of repeatability seems to be the coating of the hypodermal surface which acts as a template, in the absence of stresses that would change cell shape. The geometry of the CuP corresponds the geometry of CeP at the stage of deposition of the epicuticle (but see below for polarized epithelial cells).

3.2. Cellular processes and hypodermal growth

If the scutes are impressions of the hypodermal cells, then it is possible to read stage-to-stage variations in CuP as the result of cellular events.

3.2.1. Mitosis as the main cellular process for growth. The scute average size in the sampled areas of the median-anterior zone of the cephalic shield is 129.1 μm^2 (n = 18). We found no significant correlation between scute average size and developmental stage (r = 0.20, n = 18). Nevertheless, size variation is conspicuous among the scutes belonging even to small cuticle areas of the same individual (average Pearson's coefficient of variation, C.V. = 0.3; our calculation from unpublished data provided by M. Gamper, in litt.

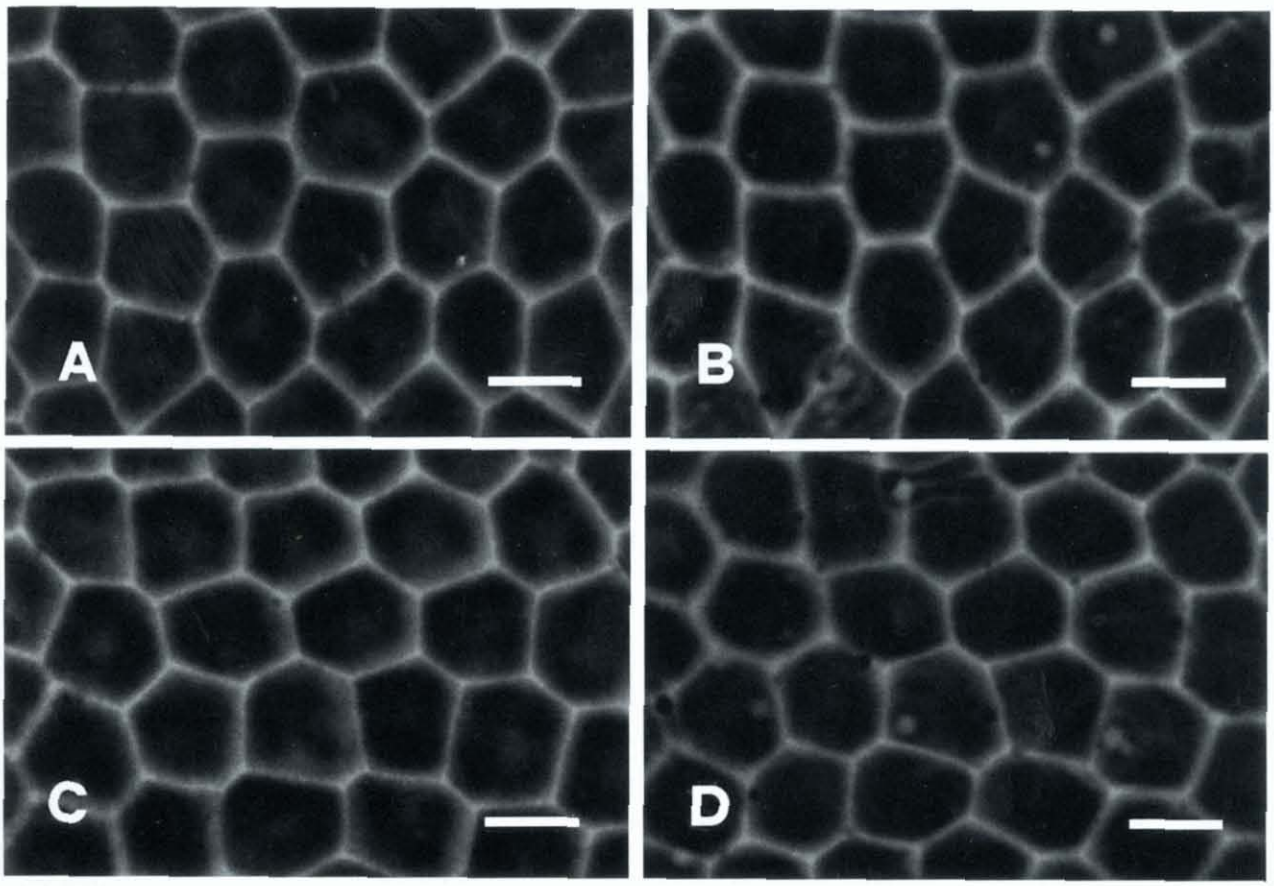

Fig. 4. Conservation of cuticular pattern in a pair of exuviae (left column vs. right column) of the same individual in subsequent (adult to adult) developmental stages; bar = 10 µm (phase contrast microscopy). **A, B:** *Lithobius muticus* (growth rate = 1.01), cephalic shield; **C, D:** same individual, another area, close to that in A–B.

1996). The main cellular process accounting for size change in integument growth is mitosis, but there are detectable size variations due to the spatial adjustment of cells, i.e. the result of each cell's changes in shape that allow it to find its place in the meshwork of the surrounding cells.

In cases of little growth, cellular events are detectable because of their isolated occurrence in areas of minor configurational change. A few examples are illustrated in Figure 5. In the first example (Fig. 5A, B), a slight variation of the polygonal pattern is due to the first appearance of a new scute in the later of the two stages.

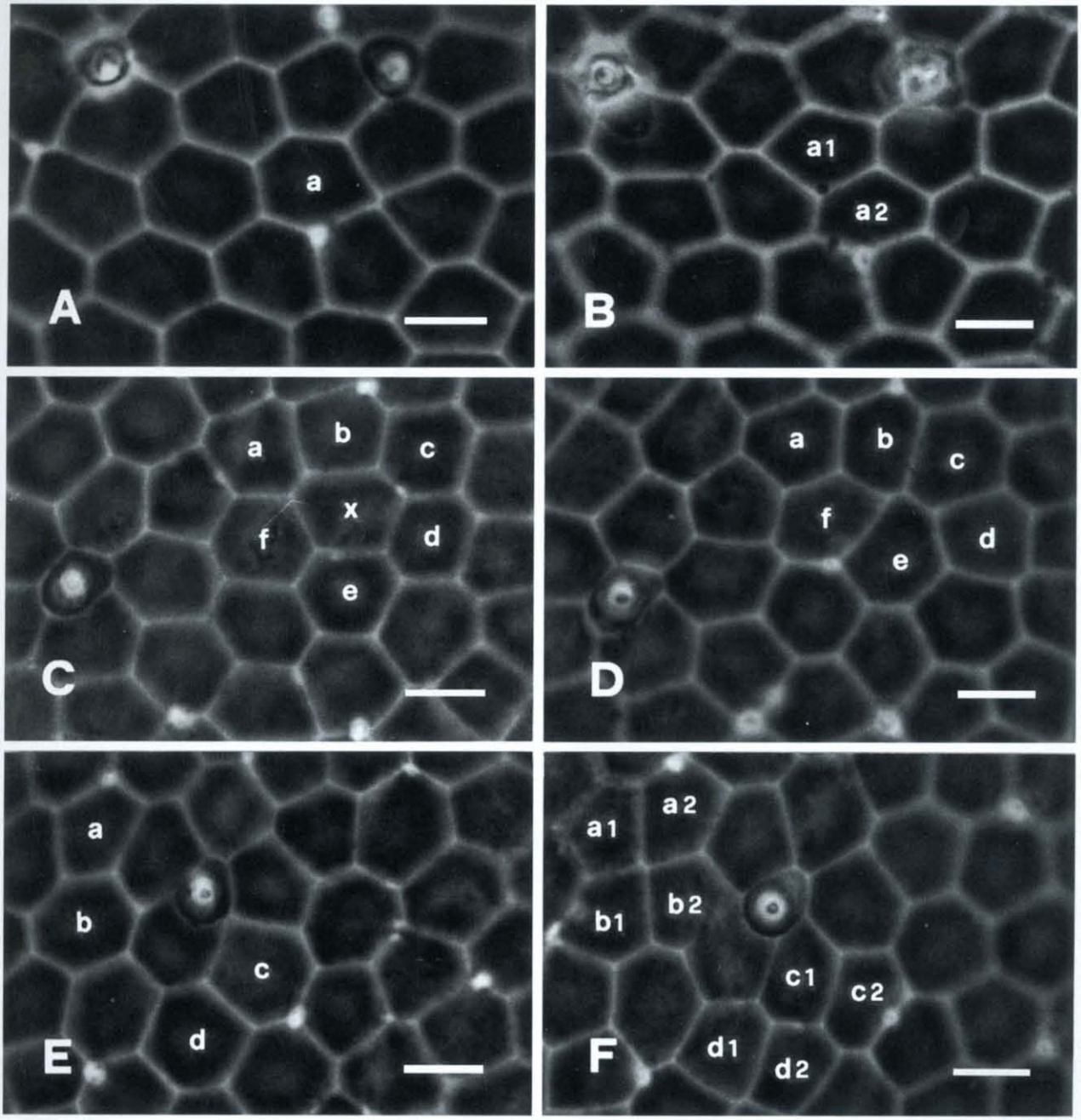

Fig. 5. Variation of cuticular pattern in pairs of exuviae of the cephalic shield of the same individual in subsequent (adult to adult) developmental stages (left column vs. right column). Polygonal areas (scutes) are interpreted as impressions of the outer faces of hypodermal cells; bar = 10 µm (phase contrast microscopy). **A, B:** *Lithobius muticus* (growth rate = 1.01); a, cell supposed to divide into a1–a2; **C, D:** *L. muticus* (growth rate = 1.06); x, cell supposed to sink or die; a, b, c, d, e, f surrounding cells; **E, F:** *L. muticus* (growth rate = 1.06); a, b, c, d, cells supposed to divide into a1–a2, b1-b2, c1–c2 and d1–d2 respectively.

For geometric reasons this is quite reasonably the result of a mitosis (note the position of the adjacent micropore, under the scute labelled *a* in Fig. 5A, then under the scute labelled *a*2 in Fig. 5B). This mitosis being isolated in an area of scarce cellular activity, its effects in deforming the previous stage's cellular pattern are easily detected. Where growth is larger (Fig. 5C–F), a more extended rearrangement of the pattern is observed. A smaller number of scutes can be identified across the two stages, but the relative position of cells, pores and (to a lesser degree) micropores allows the identification of cellular events. Figure 5E–F supports a lack of lateral inhibition on mitotic events.

3.2.2. Spatial distribution of mitoses. When the linear growth rate is in the normal range (for lithobiomorphs, on the average, between 1.08 and 1.18), the rearrangement of the tissue is so extensive that only specialized structures can be easily recognized. With a linear growth rate of 1.14, the surface growth rate is $1.14^2 = 1.30$; i.e. a 30% area increase. Even excluding cell death, an area with 100 cells of unchanged average size will became an area with 130 cells, 60 of which are "new", as the outcome of the 30 mitotic events. Therefore, in cases of normal growth, with uniform distribution of cellular events, only very small groups of scutes can actually maintain their detailed pattern from one stage to the following.

It is not possible to trace the lineage of all hypodermal cells on purely geometric evidence, but within small areas the polygonal pattern provides reliable quantitative information on the cellular events involved in hypodermal growth. Allometric analysis of the relationship between log-transformed length and width of the median-anterior zone of the cephalic shield gave a regression slope of 0.99 (SE = 0.047, n = 23). This value is not significantly different from 1 ($p > 0.9$, $n = 23$, two-sided *t*-test), which means isometry, extending to the whole cephalic shield the result of the GLS fitting in the median-anterior zone of the same sclerite. Moreover, GLS fitting showed that growth is intercalary, i.e. not confined to growing edges, at the resolution of a few tens of micrometers (the average distance between neighbouring landmarks).

To achieve intercalary isometric growth via cell multiplication, in the absence of significant changes in average cell size during ontogeny, mitoses are expected to be evenly distributed over the whole area. But a uniform distribution could be achieved, as extreme cases, either by a uniform *ordered* distribution of cellular events (where cell activity depends on the activity of the neighbouring cells) or by a uniform *random* distribution of cellular events (where each cell behaves autonomously). When large areas are considered, the results of the two processes tend to converge towards an indistinguishable uniform growth. But in small areas (dozens of cells) the random process is expected to produce local deviations from the average growth. The result of the morphometric analysis on the variation of

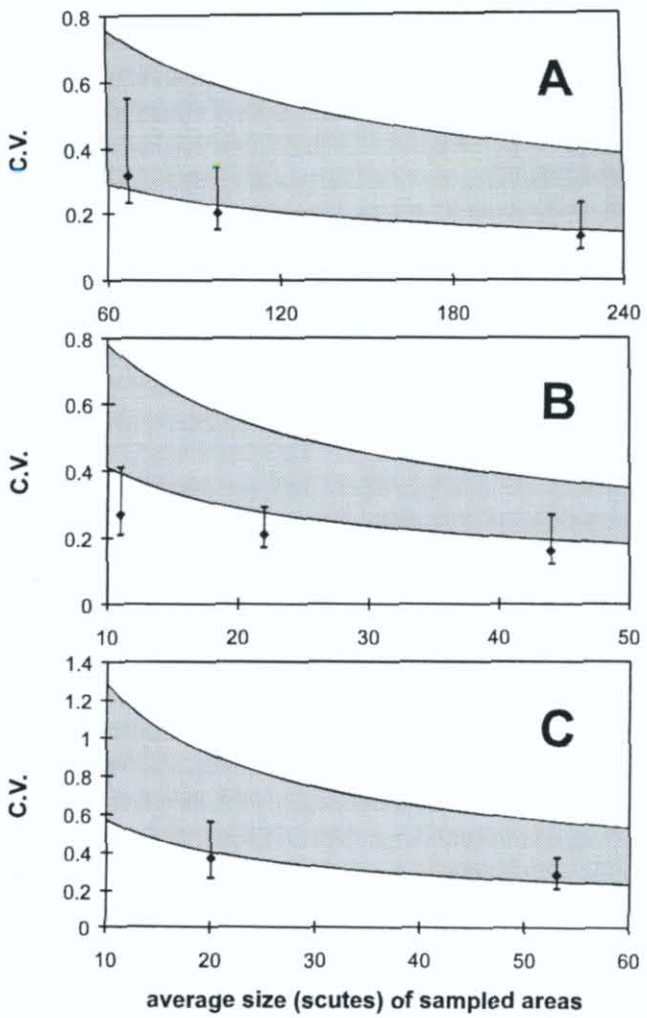

Fig. 6. Comparison between observed values of Pearson's coefficient of variation (C.V.) of quadratic growth rate and C.V. values predicted by a random model of spatial distribution of mitoses for sampled areas of different size. **A**, **B** and **C** are different specimens of *L. muticus* that vary for estimated developmental stage (numbered from the first larval, ds(a) = 11, ds(b) = 7, ds(c) = 9), quadratic growth rate (gr(a) = 1.18, gr(b) = 1.27, gr(c) = 1.20) and for organules density (organules/mm^2, od(a) = 200, od(b) = 380, od(c) = 298). Bars indicate 95% confidence interval of C.V.. The shaded belt represents the family of curves predicted by the model between two extreme hypotheses: the lowest curve represents the expected values of C.V. if mitosis alone accounts for increase in the number of cells; the upper curve represents the expected values of C.V. for the highest combination of mitosis and cell loss (see Appendix).

landmark configuration across stages are shown in Fig. 6. Here it is possible to compare the C.V. values calculated on the observed growth rates of sampled areas and the C.V. values predicted by the null model of random spatial distribution (see Appendix). All observed values are close to the lowest boundary of the family of curves predicted by the random model but slightly outside, in a zone of more ordered configurations. The mismatch with the predictions of a pure random model is reinforced by considering the probability, even a small one, of cell loss, which would lower the likelihood of the lowest curves in the family. The observed growth pattern shows a character of randomness but some spatial constraint at the level of the whole area seems to be at work.

3.2.3. Organules constrain growth. A mitotic event modifies an epidermal pattern depending on the site where it occurs and on spindle orientation, but also on the way the neighbouring cells accommodate to host the new cell pair. We think that the configuration of pre-existing pluricellular structures, like sensory or glandular cell clusters around setae and pores, restrain in some way the effects of a random distribution of mitoses by constraining the spatial adjustment of neighbouring cells. The position of setae and pores is possibly constrained by their links to internal structures (e.g. nerve fibers in the case of sensory setae), or simply by their bulkier oligocellular organization. The suggestion that organules' configuration can resist deformations caused by the distribution of mitoses is supported by the observation that the specimen with the highest density of organules (Fig. 6B, density = 380 organules/mm^2) presents the most marked deviation from the model, in the direction of more ordered patterns of growth.

4. DISCUSSION

The match we recorded between subsequent exuviae is never geometrically perfect and is easily documented only in small areas with little growth, but this is exactly what is expected when the common template underlying the two patterns under comparison is a living tissue. We demonstrated that the cuticular pattern in the cephalic shield of *L. muticus* reproduces faithfully the shape of hypodermis surface during the first deposition of the epicuticle. At that moment, the external faces of hypodermal cells are in contact with the moulting fluid and slightly compressed laterally under the old cuticle in case of recent mitoses, thus probably taking a convex shape. The very first layers of the cuticle coat the cell surface accurately, while the cuticular material deposited later from inside tends to equalize the depositional surface.

Moving cephalocaudally along the dorsal surface of the lithobiomorph trunk, a gradual transition is observed from the simple polygonal pattern discussed so far, which is based on the isotropic coating of the outer face of each cell, to a cuticular pattern of oriented scutes with some raised edges, in the most extreme cases (on most posterior tergites and also on the legs) overlapping the neighbouring scutes, as true scales (Figs. 7 and 2B). In centipedes, this pattern is usually far from conspicuous, but in other arthropods (e.g., in some isopods) such cuticular "scales" are noteworthy. Locke (1987) observed a similar anisotropic pattern on the basal surface of the hypodermis of *Calpodes* caterpillars. Here, the posterior edge of each cell overlaps the anterior edge of the cell behind it, with an overall tiled effect. One wonders whether the anisotropic pattern observed in the cuticle of our centipedes is still a unicellular template pattern, i.e. the result of coating transiently tiled cells, or a

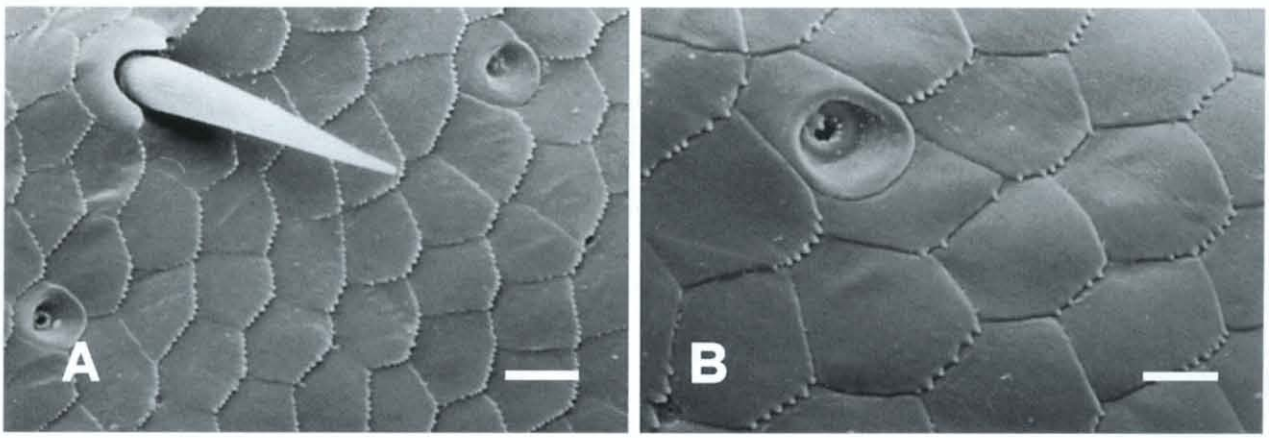

Fig. 7. Polarized cuticular scutes patterns in the cuticle (SEM microscopy). **A:** *Eupolybothrus grossipes*, 14th trunk tergite, caudal direction toward the bottom right corner, bar = 10 µm; **B:** *Lithobius forficatus*, femur of the 15th left leg, distal direction toward the bottom right corner, bar = 5 µm.

unicellular derived pattern resulting from polarized deposition and shaping of the cuticle.

There are several possible explanations for the widespread occurrence of CuP on the centipede body. The intermoult linear growth rate (the so called Dyar's value) of lithobiomorphs is on average quite low (Albert 1982). Our calculations on Andersson's (1976) data for the cephalic shield of *L. forficatus* give rates (1.09–1.18) conspicuously lower than the corresponding figures for insects at large (e.g., a rate of 1.40 as generally accepted under Dyar's rule, or 1.26 as suggested by Przibram's rule; cfr. Wigglesworth 1972). Accordingly, in *Lithobius* the hypodermis is not involved in the production of the enormous quantity of cuticle precursor that in insects is demonstrably able to modify the shape of the cell surface (Locke 1966). With such low Dyar's values, the cells should not be particularly compressed at the time of cuticulin deposition. Otherwise, extensive folding of the external plasma membrane under the old cuticle before the ecdysis is required. In the blood-sucking bug *Rhodnius prolixus* the size of the larval abdomen at about the time of deposition of the epicuticle is known to constrain the size of the abdomen of the adult (Bennet-Clark 1971). In centipedes there is no evidence of the integument being finished by deposition of superficial layers of wax and cement extruded through pore canals as in insects (Blower 1951). This explains the high water permeability of the integument, as demonstrated by Littlewood (1991) for *L. forficatus*, but this means also that the original sculpture of the epicuticle will be not obfuscated by later secretion.

The match between CuP and CeP demonstrated in this paper is promising from a technical standpoint. In those areas where a precise correspondence between the two patterns is verified, observing of CuP allows the exploration of aspects of growth and development at the cellular level. It is possible to study distribution and magnitude of basic cellular processes involved in the development of integumental structures *in vivo*, providing precious longitudinal data. Our analysis of CuPs, although limited to a small area of the cephalic shield, suggests that mitosis is the fundamental cellular process in integument growth, because of the negligible correlation between scute size and developmental stage, but also that adjustments of cell size are extensively involved in the global control of sclerite shape (high C.V.).

The uniform distribution of mitoses to achieve intercalary isometric growth is not realized via lateral inhibition. However, while mitoses seem to proceed almost autonomously, some global spatial constraint, probably due to specialized integumental structures, generates a growth pattern more ordered than that expected from a random distribution of mitotic events. In so far as the organization of the hypodermis at a given developmental stage constrains the possible changes available to the following stage, there seems to be no active shape control, at least in the case of simple isometric growth. Mitoses produce a "growth potential" exploited after the ecdysis, whose regulatory mechanism is not known (cfr. Freeman 1990).

We think that lithobiomorphs (and centipedes in general) represent good material for the study of growth at the cellular level because of the presence of good cuticular markers of cellular events in the hypodermis and the relatively low intermoult growth rate that naturally limits the number of events per moulting cycle.

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APPENDIX

The null model. We calculate the expected values of relative variation (Pearson's coefficient of variation, C.V.) of quadratic growth rate (the square of linear growth rate that accounts for areal growth) for cuticular areas of defined initial size (as number of cells), when the probability to divide is the same for all the cells, i.e, with a random spatial distribution of mitoses. Each cell is allowed to perform just one mitotic cycle. The average cell size is constant. The model takes in account the expected C.V. values for two extreme cases: a) when changes in cell number only depend on mitosis; b) when mitosis as well as cell loss are involved, but under the constraint that a single cell cannot undertake both.

Under these hypotheses, the number of mitoses (M) recorded in an area of n cells follows a binomial random distribution

$$M = Bi(n, p)$$

where n is the initial number of cells in the area and p is the probability of mitosis for a cell. The mean for a binomial random variable $Bi(n,p)$ is np and its variance is $np(1-p)$. Thus C.V., that is the ratio between standard deviation and the mean, is $\frac{\sqrt{np(1-p)}}{np}$.

Under case a), parameter p is directly estimated from the observed quadratic growth rate of the specimen: $p = \frac{g}{a} - 1$, where g is the quadratic growth rate of the cephalic shield and a is its observed variation in average cell size. Parameter a is a correction factor (generally small) so that $\frac{g}{a}$ is the observed quadratic growth rate due to mitosis.

Under case b), parameter p is estimated as $p' = \frac{g}{2\,a}$. This value represents the extreme combination of mitosis and cell loss that can account for the observed growth, given that a cell can either divide or be lost. Then C.V. is calculated as $\frac{2\sqrt{np'(1-p')}}{np'}$ because the possibility of cell loss doubles the domain of the variable.

For example, for $g = 1.30$ and $a = 1$ we have $p = g - 1 = 0.30$ and $p' = g/2 = 0.65$. The probability $p' = 0.65$ has to balance a probability of loss $l = 0.35$. $p' = 0.65$ and $l = 0.35$ are the highest values that satisfy the constraints $l + p = 1$ (a cell cannot undertake more than one cellular event) and $p' - l = p = 0.30$ (the p value producing the observed quadratic growth rate g).