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# Dorsoventral pattern formation: Morphogenesis of longitudinal coloration in *Graphosoma lineatum* (Heteroptera: Pentatomidae)

David TIETZ and Jan ZRZAVÝ

Faculty of Biological Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic

**Heteroptera, Pentatomidae, *Graphosoma lineatum*, morphology, development, color pattern formation, cryptic coloration, aposematic coloration**

**Abstract.** Postembryonic development of the adult color pattern of the shieldbug *Graphosoma lineatum* (organized as six symmetrical pairs of stripes parallel to body axis, i.e., with necessary dorsoventral positional information involved) was analysed and compared with patterns of some similar pentatomoids. All studied species, despite the probably different ecological role of their coloration (cryptic or aposematic), appear to display the same morphological regularities involved in their dorsal color pattern (three longitudinal pairs of stripes on pronotum and scutellum, more or less fused posteriorly and/or reduced). It is possible that a parallel origin of this color pattern reflects some general morphogenetic regularities whose models are discussed briefly in comparison with the well known situation in *Drosophila*.

## INTRODUCTION

The longitudinally striated red-and-black shieldbugs of the genus *Graphosoma* Laporte, 1832 (Fig. 1) entered the general developmental and molecular biological literature for the first time three years ago. Lawrence (1992: 146–147) characterized it as "a bug with checkerboard of anteroposterior and mediolateral stripes" but said that "so far, there are no models to explain this mediolateral patterning". However, the longitudinal color pattern is a mere expression of dorsoventral polarity (this fact is obscured by the strongly flattened shieldbug body form): various positional states along the dorsoventral axis are projected onto epidermis (which is, in fact, two-dimensional). Cells sharing the same dorsoventral positional information form either a middorsal (midventral) longitudinal stripe running from the anterior to posterior pole of the body (if their positional states are "1" or "0"), or a pair of parallel, bilaterally symmetrical longitudinal stripes (if their positional information is intermediate). The position of a longitudinal stripe is then determined by a dorsoventral pattern-forming system, as positions of longitudinal rows of spiracles or of limb bases, and no "mediolateral" or "bilateral" pattern-forming system (additional to those anteroposterior and dorsoventral, known from developmental studies on *Drosophila*) is necessary to generate bilaterally symmetrical pattern.

In the present paper we attempt to analyse the organization of this unusual color pattern through its complete postembryonic development, and interpret it from both developmental and evolutionary perspectives.

## MATERIAL AND METHODS

Material of *G. lineatum* for this study was collected in the Czech Republic. The larvae originated from short-term laboratory cultures (about 20–25°C, various host umbellifers). Prothetelic specimens, induced

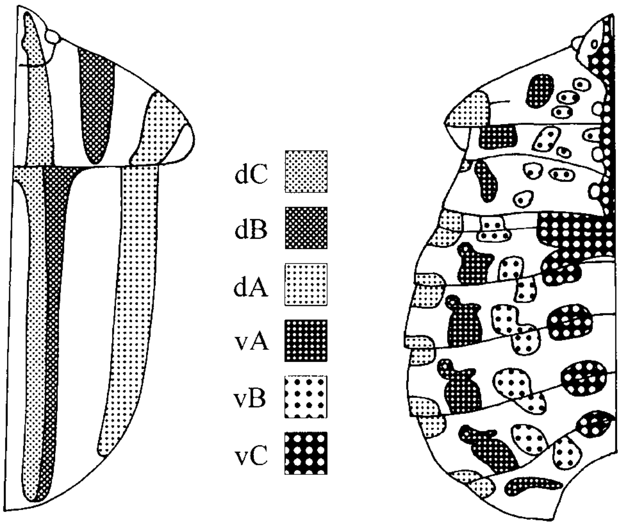

Fig. 1. Color pattern of adult *G. lineatum* and its morphological interpretation (dA–dC – dorsal rows of melanized pattern elements, vA–vC – ventral rows; white areas – non-melanized).

by JHA-treatment (Methoprene in acetone solution, 10 μg in 1 μl, topically applied), were used to reveal pattern-forming processes involved during metamorphosis of the model species. Homology of color-pattern elements in *G. lineatum* was established after comparison of specimens at various stages of postembryonic development. Morphological analysis included hot lactic acid treatment and dissections necessary for revealing the actual spatial relationships between thoracic areas.

Comparative analysis of dorsal thoracic color patterns was based on adults of 15 longitudinally striated Palaearctic species of the Pentatomoidea: Pentatomidae-Podopinae: *Graphosoma lineatum* (L., 1758); *G. rubrolineatum* (Westwood, 1837); *G. semipunctatum* (F., 1775); *Ancyrosoma leucogrammum* (Gmelin, 1789); *Tholagmus flavolineatus* (F., 1798). Pentatomidae-Pentatominae: *Aelia acuminata* (L., 1775); *A. cognata* Fieber, 1868; *A. klugi* Hahn, 1831; *A. rostrata* Boheman, 1852; *A. virgata* Klug, 1841; *Neottiglossa leporina* (Herrich-Schäffer, 1830). Scutelleridae: *Odontotarsus purpureolineatus* (Rossi, 1790); *O. robustus* Jakowlew, 1883; *Odontoscelis dorsalis* (F., 1803); *O. fuliginosa* (L., 1761).

## RESULTS

The epidermis of *G. lineatum* is almost uniformly red (or brown in larvae). The complex black-and-red color pattern is formed exclusively by different melanization of cuticular areas (red epidermis + transparent cuticle → "red", red epidermis + melanized cuticle → "black"). Therefore, only cuticular melanization will be discussed below, which is also a product of complex patterning of the epidermal cells.

### Abdomen

Major developmental changes are depicted in Fig. 2 (only hemisegments, i.e., the lateral halves of segments between the middorsal and midventral lines, are described). All larval (L) melanization is of a mosaic nature – small melanized dots (of a single-cell origin?) develop within still non-melanized cuticle, and then they fuse; however, continuous stripes running across several segments are always subdivided by non-melanized intersegmental membranes.

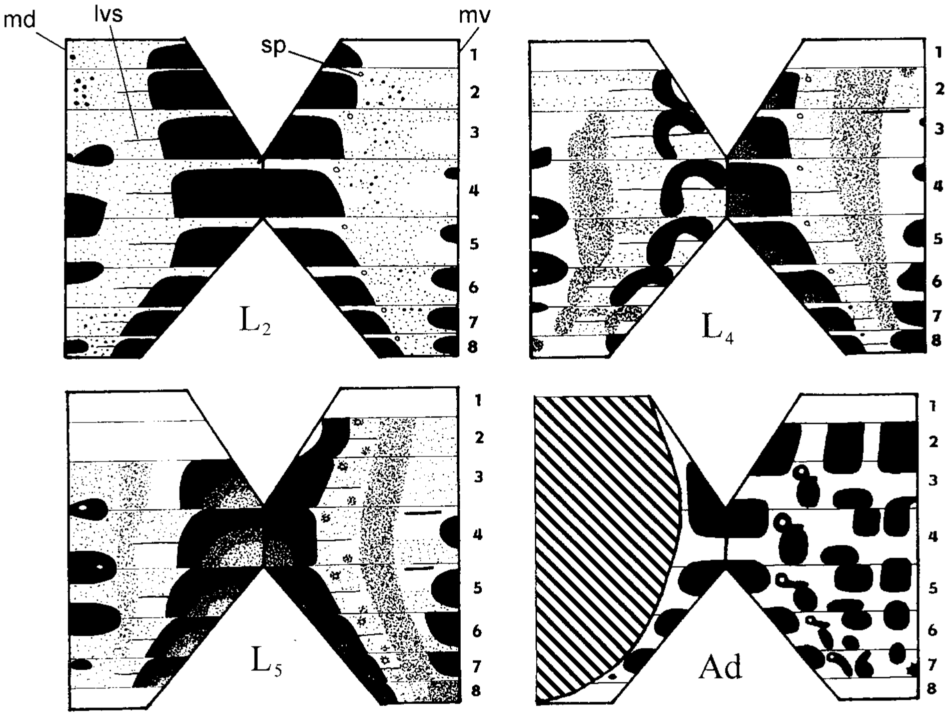

Fig. 2. Postembryonic development of abdominal color pattern in *G. lineatum* left hemisegments (1–8 – abdominal segments, L – larval instars, Ad – adult, sp – spiracle, md – middorsal line, mv – midventral line, lvs – lateroventral suture). $L_1$ and $L_3$ are similar to $L_2$-pattern. Dorsal areas covered by scutellum are not shown (they are uniformly dark).

$L_{1-3}$: Three early instars possess similar coloration: abdominal segments are non-melanized except for three rows of spots, connexival (= laterotergal), middorsal (around scent glands), and midventral (on segments 4–8). Connexival spots never touch the anterior borders of the segments (Fig. 2: $L_1$).

$L_4$: Two further longitudinal dark stripes become apparent, laterodorsal and lateroventral. Midventral spots (segments 6–8) are situated more anteriorly than in earlier instars; connexival spots acquire more complex, crescent-like shapes (their lateroposterior parts lose their melanization).

$L_5$: Similar to $L_4$; only spiracular spots are formed as well as spots in posterior parts of the ventra (between connexival and lateroventral elements).

Adult: Midventral spots are more or less subdivided along the midventral line into pairs of spots, and appear to exceed anterior segment boundaries (see Discussion). The lateroventral longitudinal stripe is subdivided into two spots, anterior and posterior, per segment; the anterior spots also exceed anterior segmental borders. Spiracular spots more or less fuse with posterolateral spots. Connexival spots lose their posterior parts and appear to spread onto preceding segments. Dorsum is uniformly dark (covered by the scutellum and thus not shown in Fig. 2: Ad).

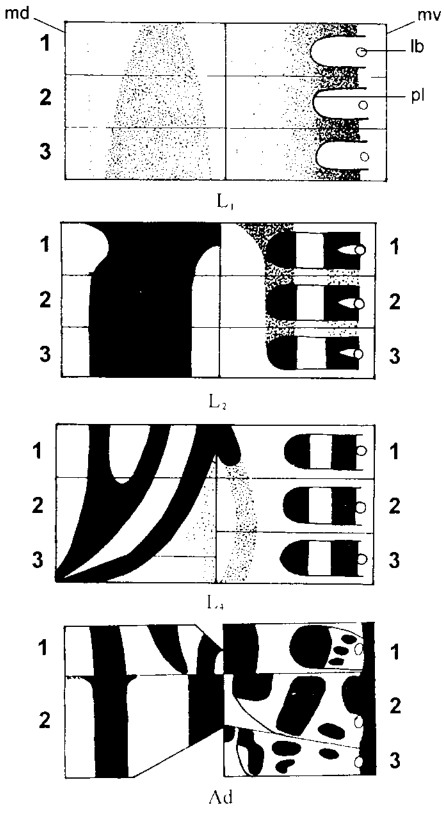

Fig. 3. Postembryonic development of thoracic color pattern in *G. lineatum* left hemisegments (1–3 – thoracic segments, L – larval instars, Ad – adult, lb – limb base, pl – pleuron). Pattern of $L_3$ is similar to $L_2$, that of $L_5$ to $L_4$). Dorsal areas covered by pronotal disc and scutellum are not shown (they are uniformly dark); adult dorsal morphology is distorted to be comparable with larval situation.

### Thorax

Interpretation of the thoracic coloration is much more complicated due to complex morphology of the thorax in insects in general and in pentatomoids with hypertrophied (meso)scutellum in particular (Fig. 3; only hemisegments are described). All larval melanization is composed of small melanized dots.

$L_1$: Thoracic melanization weakly developed, middorsal and midventral lines non-melanized.

$L_{2-3}$: Dorsum is being progressively melanized (weakly sclerotized middorsal line remains non-melanized, melanization of the lateral margin of dorsum is delayed up to $L_3$); sternopleural area non-melanized except for two dark stripes on the pleuron (Fig. 3: $L_2$).

$L_{4-5}$: The scutellum is enlarged, covering the metathorax. Pronotal and scutellar patterns are formed by three longitudinal stripes; two median stripes on each side fuse distally on the scutellum. Sternopleural areas retain the $L_3$-pattern, but a new melanized stripe appears (in the lateral, i.e., "dorsal", part of pleuron; Fig. 3: $L_4$).

Adult: Two of three scutellar stripes completely fused (Fig. 1: dC+B). Midventral line is melanized; the more ventral (coxal) of two pleural stripes is fragmented into three spots per segment (Fig. 1: vB). The areas covered by the scutellum and pronotal disc as well as major endoskeletal invaginations are uniformly melanized.

## DISCUSSION

### Color pattern organization in *Graphosoma lineatum*

The abdominal pattern is formed by pattern-forming centers organized as six longitudinal stripes per hemisegment, three dorsal (d) and three ventral (v; Figs 1–2); note that the morphological boundary between dorsum and venter is marked by the line of spiracles, and the whole connexivum (i.e., laterotergites, both "dorsal" and "ventral") belongs, in fact, to the dorsum:

(i) middorsal centers (dC): not distinct in adults (as their dorsum, covered by the scutellum, is uniformly dark);

(ii) laterodorsal centers (dB): situated between connexival and middorsal lines; they are developed in $L_{4-5}$ but indistinct in adults due to their uniformly melanized dorsum;

(iii) connexival centers (dA): they extend beyond the seeming dorsal/ventral edge and, thus, are visible also from the ventral side (Fig. 1);

(iv) centers at actual dorsal/ventral margin and its ventral vicinity (vA): i.e., spiracular spots and spots situated posteriorly to the lateroventral suture (Fig. 2) where lateral tergosternal muscles are inserted; they are developed in $L_4$–adult;

(v) lateroventral centers (vB): situated between spiracular and midventral areas: they are anteroposteriorly split during metamorphosis (this process is visible in prothetelic specimens); they are developed in $L_4$ and persist to the adult as well;

(vi) midventral centers (vC): unpaired in larvae while secondarily paired in adults (also revealed by the prothetelic specimens).

The color pattern in thorax is similar, however obscured by its complex morphology. Lateroventral spots (vB) are fragmented into three spots during metamorphosis (Fig. 1). Three paired pronotal stripes represent the original state (dA, dB, and dC) while two paired scutellar stripes are formed by the fusion of both median stripes (dA and dB+dC; Fig. 1); thoracic areas covered by the pronotum and scutellum are uniformly dark throughout all development; some thoracic color-pattern elements have different developmental timing than those abdominal (vB, vC). In the closely related *G. semipunctatum*, pronotal stripes are fragmented into two (dB) or three (dC) isolated spots; thus, it is probable that, in *G. lineatum*, pronotal stripes are formed by the activity of several anteroposteriorly aligned point pattern-forming centers (see below). No similar morphological information concerning organization of the scutellar pattern is available.

Morphogenesis of the abdominal color patterns in *G. lineatum* shows two interesting aspects:

(i) The color pattern of each abdominal segment seems to be divided into two parts, anterior and posterior. They appear to be isolated by a line confluent with the lateroventral suture and marked by the posterior margins of dA and vC spots, by the line of contact of anterior and posterior vA spots, and by the anteroposterior splitting of vB spots (see Figs 1–2). This anteroposterior intrasegmental patterning is not a consequence of the mere presence of the lateroventral suture, since this suture is structurally developed only in dA–vA area. This accords with previous results concerning intrasegmental patterning in Pyrrhocoridae and their relatives (Heteroptera) (see Zrzavý et al., 1993; Zrzavý, 1995).

(ii) The intersegmental boundary does not appear to be an insurmountable barrier for the development of individual spots, as they are able to exceed this boundary during adult metamorphosis (namely, anteriorly: vB, vC, dA). This agrees neither with other conclusions on development of heteropteran color patterns (they are always strictly intrasegmental), nor with general hypotheses about function of intersegmental boundary (as a barrier for cell lineages; see Caveney, 1985; Lawrence, 1992; Zrzavý, 1995). This unusual situation in *G. lineatum* may indicate that differentiated epidermal cells, being in contact with epidermis of the more anterior segment, are able to induce similar differentiation of these adjacent cells (cf. Nijhout & Rountree, 1995). However, another and more orthodox explanation exists: the spots do not migrate anteriorly but two independent events, posterior demelanization of one segment and posterior melanization of that more anterior, concur in the same ontogenetic stage. None of these hypotheses is supported by the color pattern of the analysed prothetelic specimens – they possess either the $L_5$-like or adult-like anteroposterior position of the vC-, vB-, and dC-spots.

### Parallel evolution of longitudinal dorsal patterns

Only adult longitudinal pronotal and scutellar patterns were examined in several pentatomoid species. All their patterns are identical with that of *G. lineatum*, or at least compatible with it (the same interpretation scheme can be used; individual color patterns are depicted in Stichel, 1960). The closely related species, *G. semipunctatum*, displays suppressed ventral thoracic melanization and its pronotal stripes are fragmented into isolated spots (for a survey of its individual variability see Stichel, 1960: all aberrations except for "f. *confluens*" are fully compatible with the present interpretation of the color pattern of *G. lineatum*). Either pronotal or scutellar stripes are fused, reduced or anteroposteriorly fragmented in some studied species. Only exceptionally (e.g., *Odontotarsus purpureolineatus*) there may be more stripes than in *G. lineatum* which would not appear to be easily explained by the present scheme. However, the *Odontotarsus* coloration is evidently derived from something like *G. lineatum*: some stripes (especially pronotal dC and dB), are more strongly melanized in their median and/or lateral margins and even tend to be split longitudinally.

However, the pentatomoid ancestor could hardly be longitudinally striated (although phylogenetic analysis of the superfamily is still lacking; for a review see Schaefer, 1993), and longitudinal color patterns certainly evolved several times in parallel (J. Vilímová, pers. commun.). If they appear to be identical, two interpretations are possible:

(i) Similar organization of the color patterns is a result of natural selection which operates on the individual species independently, forcing them to evolve in parallel; the individual patterns are not homologous. The coloration is without doubt functionally important (crypsis, aposematism, mimicry, sexual displaying, thermoregulation), and natural selection must play an important role in its evolution. However, although it is possible to expect similar color patterns in two similarly pigmented aposematic and sympatric species (they are selected to have similar display for the predators be unable to distinguish them), there is no reason to expect similar color patterns in two cryptic species (they are selected to be effectively invisible, not to be confused), or within a cryptic/aposematic couple of species.

(ii) No ecological explanation is needed if these patterns are similar because they are morphogenetically easy to evolve, as they reveal some homologous predispositions in the organization of insect body; the individual patterns are then parallel expressions of some underlying homology. This prepattern was used as exaptation for origin of aposematic coloration in *Graphosoma* spp. (*G. lineatum* larvae are rather cryptic, and the adult red-and-black aposematic coloration is undoubtedly an evolutionary novelty: J. Vilímová, pers. comm.). Consequently, the general similarity of many pentatomoid species is probably a mere consequence of a shared morphogenetic pathway: any animal with these pathways tends to respond to similar adaptive pressures (e.g., "be aposematic!") by evolving the same morphology. The color-pattern similarity may not necessarily be interpreted as a result of convergent adaptations but rather as an evidence of developmental constraint: "... identical forms can be obtained when particular developmental phenomena are triggered by very different kinds of stimuli or when constraints exist that shape external form or limit morphological expression to a few options" (Wake, 1991: 543).

Comparative ontogenetic studies of the longitudinally patterned pentatomoid species are needed to resolve this dilemma; moreover, there are also some species with longitudinal larval patterns but uniformly colored in adults (e.g., *Carpocoris* spp.).

### Possible morphogenetic consequences

It is not surprising that homologous color pattern elements can be expressed in forms of both stripes or spot rows in ontogeny (*G. lineatum*) as well as in phylogeny (pronota of *G. lineatum* and *G. semipunctatum*). Models of self-organizing morphogenetic systems (e.g., Lewis et al., 1977; Meinhardt, 1984; Nijhout, 1991; Kauffman, 1993) show that the conspicuous diversity of butterfly wing or mollusc shell color patterns can be derived from the activity of a few interregulated chemicals, and that whether the models produce striping or spotting pattern depends on their initial quantitative parameters. Moreover, Nijhout (1991: 218) suggests that the difference between line and point organizations of the pattern-forming centers is due to a simple difference in developmental timing of differentiation of these centers (i.e., due to heterochronic shifts).

Several paired longitudinal stripes in *G. lineatum* indicate that some iterative dorsoventral pattern-forming information is present. There are several known genes, both maternal and zygotic, which are involved in dorsoventral polarization and pattern formation of the *Drosophila* embryos, but no such gene is expressed in several stripes (for reviews see, e.g,. Lawrence, 1992; St Johnston & Nüsslein-Volhard, 1992). Alternatively, the iterative pattern may be explained as a result of overlapping expression domains of several different genes, none of them possessing an iterative pattern. The different timing of development of the individual longitudinal stripes appears to support the latter view.

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