Head segmentation in the embryo of the Colorado beetle  
*Leptinotarsa decemlineata* as seen with anti-en immunostaining

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**Abstract.** Segmentation in the head of the embryo of the Colorado beetle *Leptinotarsa decemlineata* is described on the basis of anti-"en" immunostaining of germ band stages. Six segmental units can be identified with this technique. Three segmental en stripes can be distinguished in the gnathal region, a weak stripe interrupted medially shows the intercalar segment rudiment, and one pair of preantennal en spots are taken to indicate a sixth segment. In the broad head lobes of the beetle the spacing of the six segmental units as demarcated by en regions is similar to that in other parts of the germ band. The results are discussed with respect to old and new data concerning the number of head segments and origin of the compound eye in insects.

**Key words:** Insect embryogenesis — *Leptinotarsa decemlineata* — engrailed — Head segmentation

**Introduction**

Most entomologists agree that the insect head consists of six segments. Five head segments can be identified in most adult insects by means of the well developed appendages labrum, antennae, mandibles, maxillae and labium. The intercalar segment between antennal and mandibular segments is reduced in adult insects, yet part of it persists as the tritocerebrum. In the embryo, however, the intercalar segment is clearly visible, e.g. in flies (Diedrich et al. 1991; Schmidt-Ott and Technau 1992), bees (Fleig and Sander 1986), grasshoppers (Patel et al. 1989) and collemboles (Tamarelle 1984). In crustaceans this segment is well developed and bears the second pair of antennae.

Contrary to this view, it has been suggested that the number of insect head segments is seven rather than six. Several authors describe, in various insects, either two pairs of vestiges of mesodermal or coelomic tissue anterior to the antennal segment (Wiesmann 1926; Miller 1940; Nair 1949; Rohrschneider 1968), or seven "morphogenetic units" in the head when analysing the pattern of malformations after injury in early embryos (Wada 1966). Chaudonneret (1984), on the other hand, found four "nervous centres" in the suboesophageal ganglion of collemboles. In *Drosophila melanogaster* the segment polarity genes engrailed (en) and wingless (wg) are expressed in two domains each anterior to the antennal segment (Schmidt-Ott and Technau 1992). In insect embryos the en active regions delimit the posterior compartment of each segment (Kornberg et al. 1985); therefore, the posterior margin of the en regions can be considered to show the segment borders. On this basis I found no clear evidence for a seventh head segment in the Colorado beetle.

**Materials and methods**

Adult beetles were collected in potato fields and kept in the laboratory on potato plants. Batches of newly laid eggs deposited on the lower side of plant leaves were collected and incubated in a moist chamber at 25°C. The sticky orange layer around each egg was removed with NaOCl in phosphate-buffered saline (PBS) saline so that only the completely translucent vitelline envelope remained. Fixation was done as described previously (Fleig 1990). To remove the vitelline envelope I rapidly exchanged the fixative with 100% ethanol. This caused the envelope to swell so that it could be removed easily with fine forceps. The antibody staining protocol was as described previously (Fleig 1990). The en antibody used in this study is a gift from PA Lawrence (Cambridge).

**Results and discussion**

In the early germ band stage of the Colorado beetle, *Leptinotarsa decemlineata*, anti-en immunostaining subdivides the head into six segmental units. Three gnathal stripes show the posterior compartments of the labial, maxillary and mandibular segments. One pair of antennal stripes can be identified due to their oblique orientation. One pair of spots consisting of 3-5 cells with la-
Fig. 1. Ventral view of the anterior part of the head of the Colorado beetle during germ band extension. The mandibular and antennal *engrailed* (*en*) stripes are already well seen. In between of them, in the anlage of the intercalary segment, *en*-protein is not yet detectable. A pair of *en*-spots consisting of three to five labelled nuclei is apparent anterior to the antennal stripes in the broad head lobes (arrowheads). The labelled nuclei at the rims of the head lobes belong to the amnion. *A*, Antennal segment; *I*, intercalary segment; *P*, preantennal segment; *Md*, mandibular segment; *Mx*, maxillary segment; *scale bar*, 0.1 mm

Fig. 2. Ventral view of the anterior part of the head of a young extended germ band stage. The antennae begin budding. The intercalary *en* spots are still very weak (arrow). Abbreviations as in Fig. 1. *Scale bar*, 0.1 mm

In the extended germ band stage of the Colorado beetle the anterior part of the head grows larger and mouth and head appendages become visible morphologically (Figs. 2, 3). The *en* spots in the intercalary segment rudiment appear but they remain weaker than the other *en* stripes and spots. The anlage of the intercalary segment is of normal size in the Colorado beetle (Figs. 1–3) as well as in the honeybee (Fleig et al. 1992) when compared with the segment anlagen in other parts of the embryo. In older germ band stages of the beetle some anti-*en* label can be seen in the developing clypeolabrum; however, this label remains weak and seems to be not strictly nuclear (Fig. 3). No further *en* signals appear in the embryo head. During the extended germ band stage nuclei of the amnion cells show strong anti-*en* label (Figs. 1, 2). After germ band retraction, when the amnion has turned from the ventral to dorsal side, its nuclei no longer show anti-*en* label (data not shown).

In most textbooks the compound eyes of insects are assigned to the acron or to a single preantennal segment because of their innervation from the protocerebrum (e.g. Snodgrass 1935). Recent paleontological findings assign the insect’s eyes to the mandibular segment in a six-segmented head (Kukalová-Peck 1992). However, data concerning the embryonic head of *Drosophila* (Turner and Mahowald 1979; Jürgens et al. 1986; Chadwick and McGinnis 1987; Diederich et al. 1991), *Apis* (Fleig 1990; Fleig and Sander 1986; Fleig et al. 1992), *Schistocerca* (Patel et al. 1989) and *Leptinotarsa* (Figs. 1, 2, 3) do not support a mandibular but rather a preantennal origin of the eyes.

In the head of the fruitfly embryo Diederich et al. (1991) found an additional *en* region located anterior to the preantennal spot. The authors associate this small anteriormost *en* region with the nonsegmental acron, thus ending up with six head segments. Schmidt-Ott and Technau (1992) associate this region with the ocular segment, and they suggest an additional seventh segment in the insect’s head between labral and antennal segments; on the basis of this concept they postulate a curved sequence of head segments which would explain the location of the eye anlagen lateral to the antennal segment in the germ band stage of the fruitfly. However, labelled nuclei anterior to the antennal segment can be assigned to a preantenonal segment. In this early stage no intercalary anti-*en* label is seen, but the existence of this rudimentary segment anlage becomes clear when comparing the large distance between mandibular and antennal *en* stripes with the much shorter, rather uniform distances between the other segmental *en* stripes or spots (Fig. 1).

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