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Arthropod Relationships

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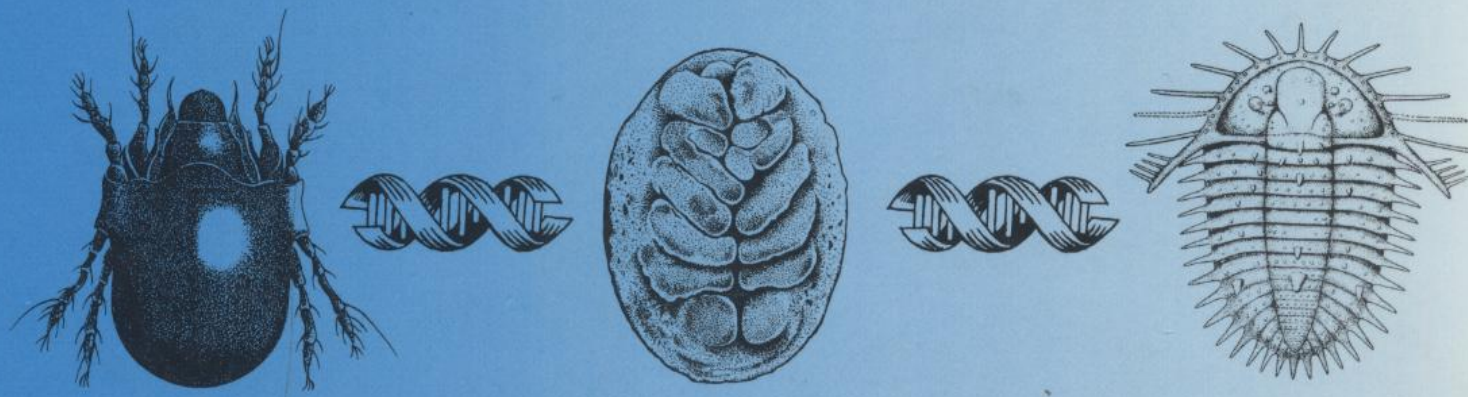

CHAPMAN & HALL

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24 Cleavage, germ band formation and head segmentation: the ground pattern of the Euarthropoda

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24.1 INTRODUCTION

24.1.1 EMBRYOLOGY AND ARTHROPOD PHYLOGENY

Comparative embryological studies have always played an important role in the interpretation and understanding of arthropod origins and phylogeny (Bowler, 1994). Embryonic data have been used to support both arthropod monophyly (Weygoldt, 1979, 1986) as well as polyphyly (Anderson, 1973). Now there is convincing and increasing evidence from palaeontological, embryological, morphological, and molecular studies that arthropods are monophyletic (Weygoldt, 1986; Wägele, 1993; Walossek, 1993; Wheeler *et al.*, 1993; Friedrich and Tautz, 1995; Wills *et al.*, 1995; Wägele and Stanjek, 1995; Garey *et al.*, 1996). This is especially clear for the 'true' arthropods, the Euarthropoda, which include the chelicerates, crustaceans, myriapods, and insects. The method of phylogenetic systematics (Hennig, 1950, 1966) allows not only the analysis of the phylogenetic relationships between taxa but it also offers a tool for the reconstruction of the ground pattern of a given monophyletic group (Ax, 1987). This ground pattern is inferred on the basis of the phylogenetic systematics and the character distribution in that monophyletic group. It represents the set of characters which were present in the group's stem species. The ground pattern is a mixture of apomorphic and plesiomorphic characters of the taxon under investigation. The knowledge of the ground pattern provides us with the starting point for the analysis of evolutionary alterations (Lauterbach, 1980; Bitsch, 1994; Sandeman and Scholtz, 1995; Scholtz, 1995a,b). The phylogenetic relationships between the higher euarthropod taxa are not settled. In particular, the close relationship between insects and myriapods has been challenged by recent investigations (Averof and Akam, 1995; Friedrich and Tautz, 1995; Osorio *et al.*, 1995; see other contributions in this volume). Nevertheless, it is possible and worthwhile to reconstruct the ground pattern of euarthropods with regard to the cleavage type, the mode of germ band formation, and the segmentation of the head. In addition, some embryonic characters are discussed that provide further arguments for arthropod and euarthropod monophyly.

24.1.2 THE SISTER GROUP OF ARTHROPODS

It is a widespread view that annelids and arthropods are closely related or, depending on annelid monophyly, even sister groups (Lauterbach, 1980; Weygoldt, 1986; Brusca and Brusca, 1990; Wheeler *et al.*, 1993; Nielsen, 1995; Westheide, 1996). On the other hand, this close relationship has been disputed and a monophyletic origin of animals with a trochophora larva (Eutrochozoa) has been suggested (Eernisse *et al.*, 1992, Winnepenninckx *et al.*, 1995; Eernisse, 1997, this volume). However, the assumption of a close relationship between annelids and arthropods is supported by a number of derived characters shared by annelids and arthropods: an ectodermal and mesodermal growth zone anterior to a terminal body portion bearing the anus, segment formation in an anteroposterior sequence with the ventral side showing a higher degree of differentiation than the dorsal side, segmental paired ganglia forming a ladder-like central nervous system, segmental paired coelomic sacs, segmental paired metanephridia, external annulation, a long dorsal tube-like heart, and mushroom bodies characterized by a certain cell type in a characteristic arrangement situated in the anterior part of the brain (Strausfeld *et al.*, 1995). The characters related to segmentation, in particular, form a complex that cannot be found in any other protostome group. On the molecular level this is supported by the similar mode of dual *engrailed* gene expression during early segmentation and neurogenesis in annelids and arthropods (Weisblat *et al.*, 1993). Thus, segmentation in annelids and arthropods is homologous and most likely an apomorphy for the Articulata. Alternatively, according to the eutrochozoan hypothesis one has to assume that either segmentation of annelids and arthropods is convergent (which is unlikely) or the common ancestor of eutrochozoans and arthropods was already segmented (Eernisse *et al.*, 1992).

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The latter assumption needs to explain the independent loss of segmentation in most spiralian taxa and it seems more plausible to interpret the serially arranged characters in some molluscs as either a first step towards the articulate-like segmentation complex (Nielsen, 1995) or as an independent development (Lauterbach, 1983, Haszprunar and Schaefer, 1996)).

24.2 CLEAVAGE

24.2.1 THE CLEAVAGE MODES IN EUARTHROPODS

Euarthropods exhibit a great variety of different cleavage types (Siewing, 1969; Fioroni, 1970; Anderson, 1973) (Figures 24.1–24.4). The common mode of superficial cleavage is characterized by yolky eggs with intralecithal cleavage divisions with the cleavage products (energids) not being separated by membranes. Later, the energids migrate to the egg surface and form the blastoderm with a central yolk mass and the germ becomes cellular (Figure 24.4). In some taxa we find a holoblastic cleavage type with a coeloblastula (Figure 24.3) and an invagination gastrula. In addition, there are cleavage modes that are intermediate between these two extremes (mixed cleavage, see Fioroni, 1970), e.g. they start with total cleavage and switch in later stages to the superficial mode showing a blastoderm stage. This variety of cleavage types is found in each of the major euarthropod groups: chelicerates, crustaceans, myriapods and insects. Even in closely related taxa several cleavage types occur and it is apparently not a big step to alter a superficial cleavage into holoblastic cleavage and vice versa. This character distribution makes it difficult to reconstruct the ground pattern of the early development of euarthropods. Contradictory views include the opinion that superficial cleavage in combination with a yolky egg is a synapomorphy of onychophorans and euarthropods (Weygoldt, 1986), while it has also been suggested that the plesiomorphic condition for arthropods is holoblastic cleavage with eggs which are poor in yolk (Siewing, 1969). The claim that this holoblastic cleavage is still a type of spiral cleavage or that it at least shows traces of the spiral cleavage pattern found in annelids (Anderson, 1973; Nielsen, 1995) has been challenged by Siewing (1969) and Dohle (1989).

The variety of combinations of developmental processes evident in the mixed cleavage types shows that early development is a sequence of stages where each step can be evolutionarily altered without affecting subsequent stages (Scholtz and Dohle, 1996). For instance, early intralecithal divisions are changed towards total divisions but the blastoderm stage is retained. Thus, it is not possible to infer cleavage mode as a whole from individual stages. We cannot conclude that a large amount of yolk leads to intralecithal divisions and that these lead to a blastoderm stage. Accordingly, these cleavage modes are subdivided into different stages which will be discussed separately.

(a) Early cleavage – intralecithal or total, radial or spiral?

The problem of reconstructing the original arthropod pattern of early cleavage is due to the difficulties in homologizing intralecithal cleavage as such. One needs certain distinct characters such as position or lineage of the division products to claim homology; otherwise, it is just the absence of membranes separating the blastomeres that unifies intralecithal cleavage. There are very few studies which have tried to trace the lineage of the early cleavage products in intralecithal cleavages (Dohle, 1970). In several euarthropod lineages intralecithal cleavage has been altered towards total cleavage and vice versa. Therefore, a different path is pursued here – cases of total cleavage are compared in order to look for more detailed similarities. With the help of additional data the question of the original euarthropod cleavage type is readdressed.

The early total cleavage of representatives of chelicerates (Pantopoda: Dogiel, 1913), crustaceans (Cladocera: Kühn, 1913; Cirripedia: Anderson, 1965; Copepoda: Fuchs, 1914; Ostracoda: Weygoldt, 1960; Euphausiacea: Taube, 1909; Decapoda: Zilch, 1978; Hertzler and Clark, 1992; Isopoda: Strömberg, 1971; Amphipoda: Rappaport, 1960), myriapods (Symphyla: Tiegs, 1940; Pauropoda: Tiegs, 1947; Diplopoda: Dohle, 1964), and insects (Collembola: Claypole, 1898) show some distinct similarities (Figures 24.1 and 24.2). They undergo an early 'radial' cleavage with mostly no oblique spindle orientation, regardless of whether the cleavages are equal or unequal (Figures 24.1 and 24.2). Subsequent divisions show mainly spindle directions orthogonal to the preceding ones, although there is some variation in this. In addition, the relative positions of blastomeres vary between individual eggs. Thus, cleavage appears irregular and no traceable cell lineage exists in advanced stages. This has been reported for several crustaceans (Weygoldt, 1960; Benesch, 1969; Scheidegger, 1976) and myriapods (Tiegs, 1940, 1947; Dohle, 1964) (Figure 24.1). The same pattern can be seen in clear cases of secondary early total cleavage as in parasitic isopods (Strömberg, 1971) (Figure 24.1) and even in intralecithally cleaving eggs (Dohle, 1970). The general pattern of early cleavage allows the conclusion that 'radial' cleavage is part of the arthropod ground pattern. The term 'radial' cleavage is used in the meaning of a radially oriented position of the cleavage products (energids or cells); it is not meant in the strict definition for the radial cleavage type given by Siewing (1969, 1979). Since arthropods are part of the Spiralia, the 'radial' cleavage is an apomorphy of arthropods. This conclusion relates only to the relative position of the division products of early cleavage. It does not clarify the question as to whether the radially oriented nuclei and the cytoplasm were separated by membranes or not.

Several authors have claimed that spiral cleavage occurs in some crustaceans with mixed or holoblastic cleavage modes (Taube, 1909; von Baldass, 1941; Anderson, 1969, 1973;

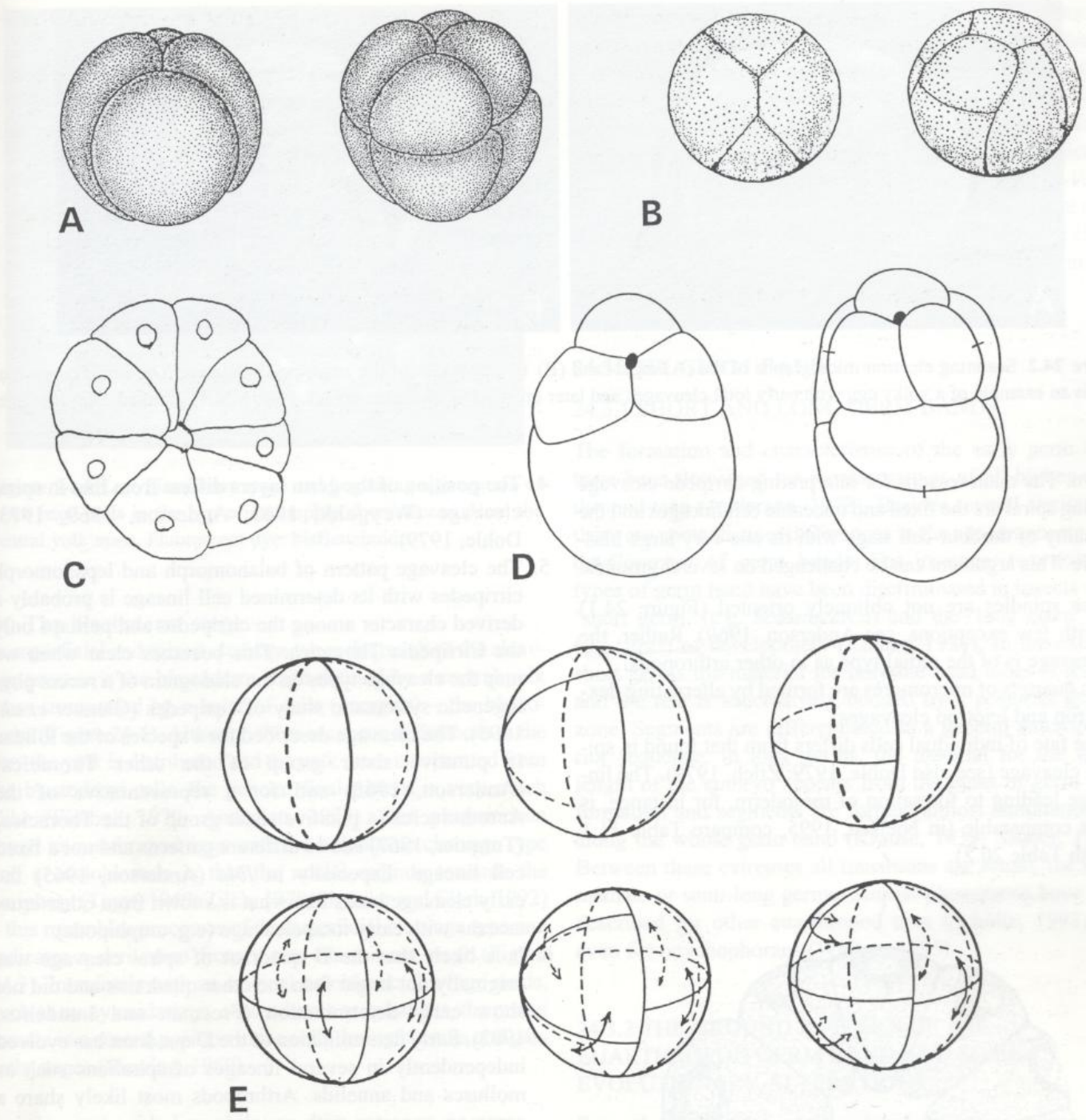

Figure 24.1 The early total cleavage of some arthropods showing the pattern of irregular 'radial' cleavage. (A) Insecta, Collembola, four- and eight-cell stages. (Modified from Anderson, 1973, after Claypole, 1898.) (B) Myriapods, Diplopoda, four- and irregular eight-cell stages. (After Dohle, 1964.) (C) Chelicerata, Pantopoda, section through a 32-cell stage. (After Dogiel, 1913.) (D) Crustacea, Cirripedia, four- and eight-cell stages. (Modified from Nielsen, 1995, after Delsman, 1917.) (E) Three different arrangements of the blastomeres in the four- and eight-cell stages of the parasitic isopod *Bopyroides*. (After Strömberg, 1971.)

Nielsen, 1995). This has been disputed by Siewing (1979), Dohle (1979, 1989), Zilch (1979) and Hertzler and Clark (1992). In particular, the cleavage patterns found in some species of Cirripedia have been interpreted in this way (Bigelow, 1902; Delsman, 1917; Anderson, 1973; Costello and Henley, 1976; Nielsen, 1995). However, these interpretations differ considerably with regard to the application of spiral nomenclature and fate of individual cells (compare Anderson, 1973; Costello and Henley, 1976; Nielsen, 1995) and cell lineages have never been analysed up to the formation of germ layers. Nielsen (1995) even considered the cirripede cleavage pattern as part of the euarthropod ground

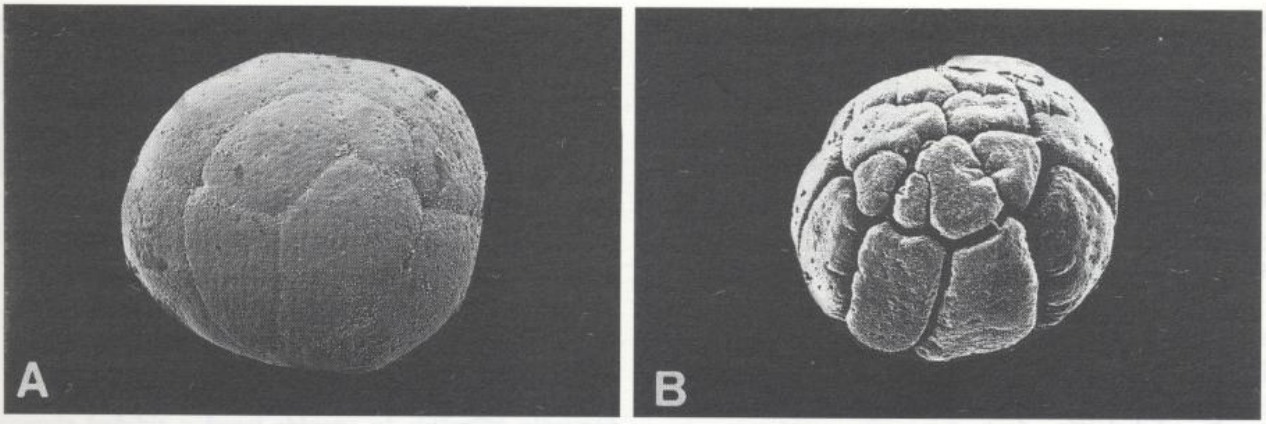

Figure 24.2 Scanning electron micrographs of the (A) eight- and (B) 16-cell stages of the amphipod crustacean *Orchestia cavimana*. This is an example of a yolky egg with early total cleavages and later on superficial characters (cf. Figure 24.4) – mixed cleavage type.

pattern. The main reasons for interpreting cirripede cleavage as being spiral are the fixed and traceable cell lineages and the inequality of the four-cell stage with its one very large blastomere. This argument can be challenged on several grounds:

1. The spindles are not obliquely oriented (Figure 24.1) (with few exceptions, see Anderson, 1969). Rather, the cleavage is of the radial type as in other arthropods.
2. No quartets of micromeres are formed by alternating dexiotrop and leiotrop cleavages.
3. The fate of individual cells differs from that found in spiral cleavage (see also Dohle, 1979; Zilch, 1979). The lineage leading to formation of mesoderm, for instance, is not comparable (in Nielsen, 1995, compare Table 11.1 with Table 20.2).

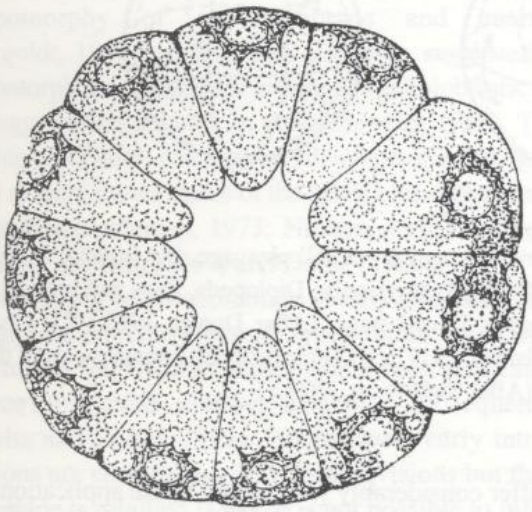

Figure 24.3 Coeloblastula of a penaeid crustacean. (After Zilch, 1979.) Although this is a holoblastic cleavage mode, the nuclei and the cytoplasm migrate towards the periphery as in the blastoderm stage of superficial cleavages (cf. Figure 24.4).

4. The position of the germ layers differs from that in spiral cleavage (Weygoldt, 1963; Anderson, 1969, 1973; Dohle, 1979).
5. The cleavage pattern of balanomorph and lepadomorph cirripedes with its determined cell lineage is probably a derived character among the cirripedes and perhaps only the Cirripedia Thoracica. This becomes clear when we map the cleavage types on the cladogram of a recent phylogenetic systematic study of cirripedes (Glenner *et al.*, 1995). The cleavage described for a species of the Iblidae (a putative sister group of the other Thoracica) (Anderson, 1965) and for a representative of the Acrothoracica (a putative sister group of the Thoracica) (Turquier, 1967) shows different patterns and not a fixed cell lineage. Especially in *Ibla* (Anderson, 1965) the early cleavage looks like what is known from other crustaceans with early total cleavage (e.g. amphipods).
6. It is likely that the D quadrant of spiral cleavage was originally not larger than the other quadrants and did not show early determination (Freeman and Lundelius, 1992). Early determination of the D quadrant has evolved independently in several lineages of spiralians such as molluscs and annelids. Arthropods most likely share a common ancestor with annelids and this stem species was then devoid of a large D quadrant. Thus, the yolky cell in cirripedes cannot be derived from an annelid-like spiral cleavage.

However, accepting that arthropods and annelids share an ancestor which was a spiralian it is sensible to look for traces of spiral cleavage in arthropod development. Representatives of the arthropod ancestral lineage must have had spiral cleavage, but in the cleavage patterns of recent arthropods there have to be at least some similarities to claim homology with spiral cleavage and this does not seem to be the case in the cirripede and other arthropod cleavage patterns (see also Dohle, 1979, 1989).

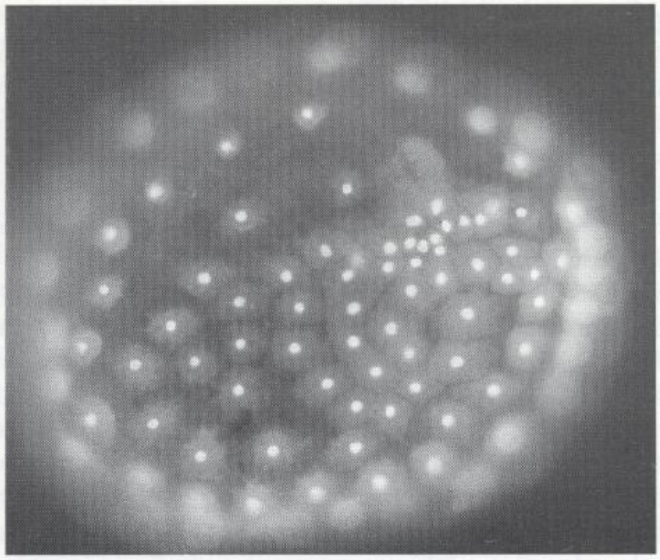

Figure 24.4 Late blastoderm stage and beginning aggregation of germ band cells in the egg of an amphipod crustacean. Note the central yolk mass. Fluorescent dye: bisBenzimide.

(b) The blastoderm stage

In superficial and mixed cleavage types the result of the cleavage process is the blastoderm stage defined by a central yolk mass surrounded by a layer of cells (energids) – the blastoderm (Figure 24.4). Although there are some differences in the way the yolk is distributed and the yolk mass is separated from the blastoderm cells, the general final pattern is very much alike (Fioroni, 1970; Anderson, 1973). One phenomenon found in euarthropod eggs with the holoblastic cleavage type and a coeloblastula is that the nuclei migrate towards the periphery (Tiegs, 1940; Zilch, 1978; Hertzler and Clark, 1992) – this resembles processes of the superficial cleavage type and indicates a derivation from a true blastoderm stage (Figure 24.3). In animals with an original holoblastic cleavage mode, such as many cnidarians, echinoderms or spiralians, the nuclei remain in a central position in the cells during blastula and gastrula stages (Siewing, 1969).

24.2.2 THE GROUND PATTERN OF EUARTHROPOD CLEAVAGE

One can reconstruct the ground pattern of early cleavage in euarthropods as follows: it was a 'radial' cleavage with some irregularities concerning spindle orientation and the relative position of blastomeres and no stereotyped cell lineage. Its widespread occurrence among euarthropods suggests that early cleavage was intralecithal. This suggestion is also supported by the fact that onychophorans originally show intralecithal cleavages (Anderson, 1973). However, the possibility cannot be ruled out that early cleavage in euarthropods was total, and I do not see a way to settle this question beyond doubt. Originally the cleavage process (intralecithal or total) led to a blastoderm with a central yolk mass. The blastoderm stage suggests a relatively yolky egg in the ground pattern of euarthropods. This is again supported by the blastoderm stages of the yolky onychophoran eggs (Anderson, 1973). Furthermore, new findings of putative trilobite eggs from the Middle Cambrian exhibiting a blastoderm stage are indicative of yolky eggs in early euarthropods (Zhang and Pratt, 1994). In summary, it is obvious that early development of the euarthropod stem species followed the superficial or mixed cleavage mode. Holoblastic cleavage modes with eggs poor in yolk are apomorphic among euarthropods.

24.3 THE GERM BAND

24.3.1 SHORT AND LONG GERM BANDS

The formation and characteristics of the early germ band have been described for representatives of all higher euarthropod taxa (Anderson, 1973). Besides several similarities there are some distinct differences in the appearance and the development of germ bands. For instance, two extreme types of germ band have been discriminated in insects – the 'short germ' (e.g. *Schistocerca*) and the 'long germ' type (e.g. *Apis*) of development (Krause, 1939). In the extreme short germs the material for only the head lobes is formed and the rest is successively budded by a posterior growth zone. Segments are differentiated in a general anteroposterior sequence. In long germs, the material for the whole length of the embryo appears from the onset of germ band formation and segments are formed almost simultaneously along the whole germ band (Krause, 1939; Sander, 1983). Between these extremes all transitions are found, the intermediate or semi-long germs. Similar phenomena have been described for other euarthropod taxa (Scholtz, 1992) and even for onychophorans (Walker, 1995).

24.3.2 THE GROUND PATTERN OF THE EUARTHROPOD GERM BAND AND SOME EVOLUTIONARY ALTERATIONS

From the distribution of germ band characters among euarthropods the following ground pattern is reconstructed. The early germ band is formed by aggregation of blastomeres on the prospective ventral side of the embryo (Figure 24.4). The anterior end is characterized by the paired semicircular head lobes that give rise to the lateral eyes and the lateral parts of the protocerebrum (Figures 24.5 and 24.6). Only the material for a few anterior segments is present. The rest is produced by the activity of a growth zone. It is likely that the original euarthropod germ band was neither of the extreme short type as in *Schistocerca*, where only the head lobes and the growth zone are present, nor a long germ type. This is deduced from the following observations. An intermediate germ has been suggested as the original state for insects because this is found in

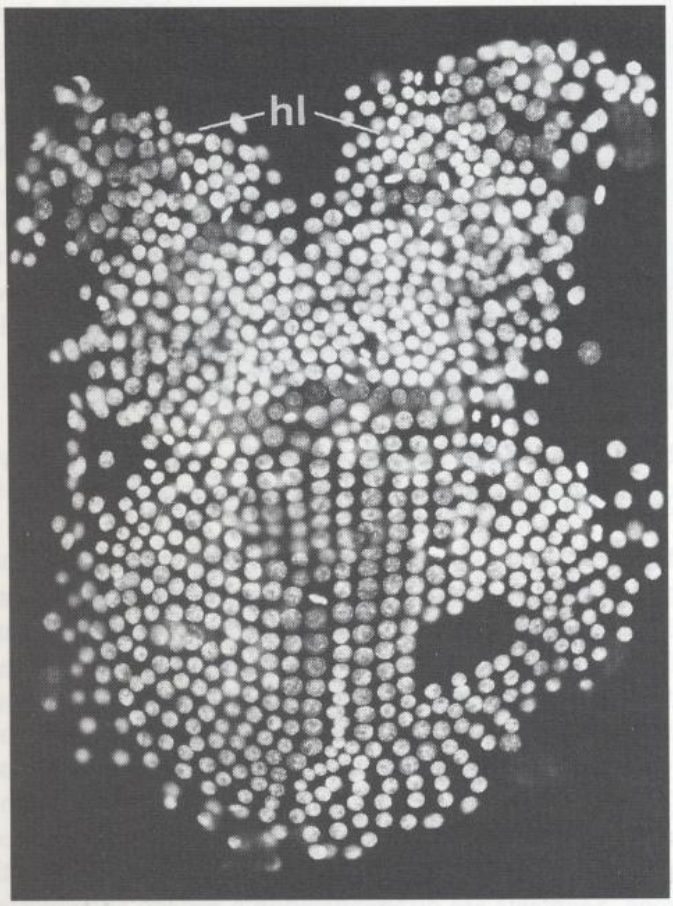

Figure 24.5 Early germ band of an amphipod crustacean (*Gammarus roeselii*) showing the anterior head lobes (hl). Fluorescent dye: bisBenzimide.

apterygote insects and representatives of many higher taxa of pterygote insects (Tautz *et al.*, 1994). Futhermore, neither crustaceans nor chelicerates possess an early germ band that comprises only the head lobes and a growth zone – the crustacean nauplius larva, for instance, consists of at least three segments and the posterior growth zone. In contrast to what is found in annelids, the growth zone of the original euarthropod germ band was not characterized by specialized cells such as teloblasts (Figure 24.7). It seems likely that the elongation of the germ band was caused by scattered irregular divisions along the germ band since Patel (1993) describes this for a short germ insect and Gerberding (1997) for a non-malacostracan crustacean (Figure 24.7). Originally, the differentiation of segments such as limb buds and ganglion formation follows a general anteroposterior sequence (Figures 24.6–24.8). This is inherited from the common ancestor of annelids and arthropods and vestiges of this process can even be found in extreme long germs like *Drosophila* (Karr *et al.*, 1989). However, the anteriormost segment is not necessarily the one in which differentiation starts ('Differenzierungszentrum'; Seidel, 1975). It can be either the mandibular segment, or the maxillary segment or the first trunk segment (Patel *et al.*, 1989; Fleig, 1990; Scholtz *et al.*, 1994; Manzanares *et al.*, 1996). It is not clear what the situation was in the ground pattern. The segment-polarity gene *engrailed* showed a characteristic pattern in the germ band of the euarthropod stem species (Figures 24.8–24.11). In the germ band of insects and crustaceans, it is expressed in iterated transverse stripes in the posterior portion of each developing segment comprising the neurogenic region and limb buds (DiNardo *et al.*, 1985; Patel *et al.*, 1989; Scholtz

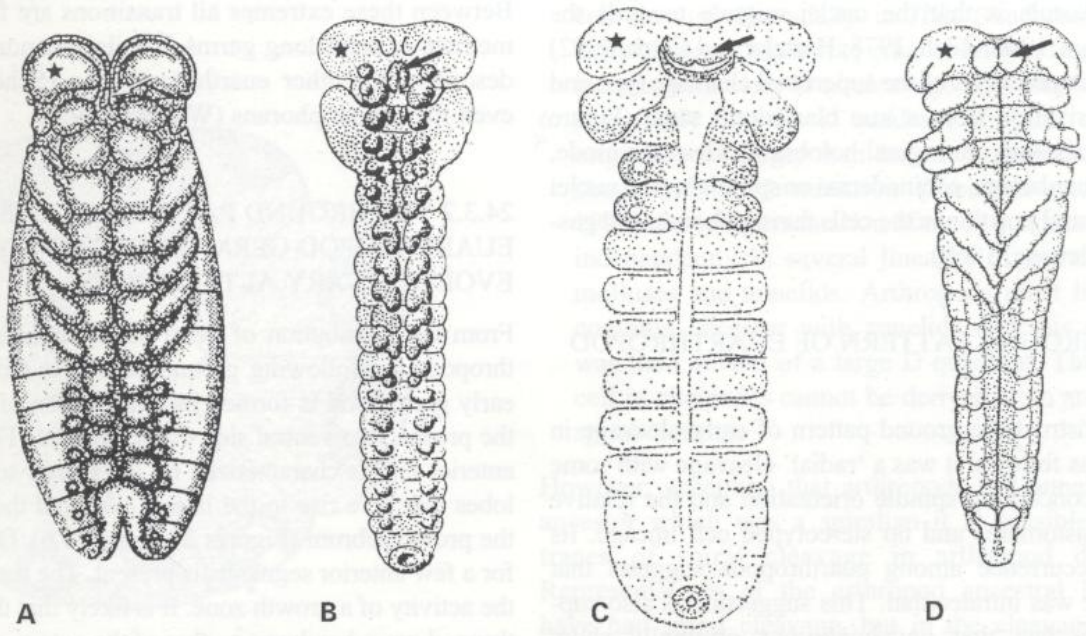

Figure 24.6 Comparison of advanced germ bands of (A) Scorpion (chelicerate) (after Brauer, 1895); (B) Isopod (crustacean) (modified from Kaestner, 1967 after Silvestri); (C) Chilopod (myriapod) (after Hertzel, 1984); (D) Caddisfly (insect) (after Kobayashi and Ando, 1990). Note the overall similarity. All germ bands are characterized by the head lobes (star) the paired labral rudiment (arrow), and the anteroposterior decline of differentiation.

et al., 1993, 1994; Brown *et al.*, 1994; Patel 1994; Schmidt-Ott *et al.*, 1994; Manzanares *et al.*, 1996; Dohle, 1997, this volume) (Figures 24.8–24.11). A corresponding *engrailed* expression pattern occurs in annelids (Lans *et al.*, 1993). It is likely that *engrailed* already played a role in segmentation in the common ancestor of annelids and arthropods. The similarity of the *engrailed* expression in insects and crustaceans conflicts with the theory that crustacean segments and biramous appendages are the result of the fusion of two adjacent original segments (Emerson and Schram, 1990; Schram and Emerson, 1991). There is now enough evidence to show that this 'duplo-segment' hypothesis is not well founded (Zrzavý and Štys, 1994; Panganiban *et al.*, 1995; Scholtz, 1995c).

Various aspects of this ground pattern have been altered in several euarthropod lineages. A long germ has been independently evolved in different taxa (Scholtz, 1992). In malacostracan crustaceans (perhaps also in cirripedes, Anderson, 1973) the growth zone underwent changes that are convergently similar to the situation in annelids (clitellates). In this group, teloblasts have evolved that lie in front of the telson and give rise to the material for posterior segments by unequal divisions (Dohle and Scholtz, 1988; Scholtz and Dohle, 1996) (Figure 24.7). This teloblastic growth is correlated with a unique stereotyped cell division pattern during germ band growth and differentiation (Dohle and Scholtz 1988) (Figure 24.7). In some spiders (Anderson, 1973), scolopendromorph centipedes (Whitington *et al.*, 1991), and amphipod crustaceans (G. Scholtz, unpublished results) the germ band is split along its longitudinal axis. The two lateral halves separate and fuse again in a later stage. In some crustaceans it is questionable whether there is an early germ band stage at all. In penaeid decapods, for instance, the germ

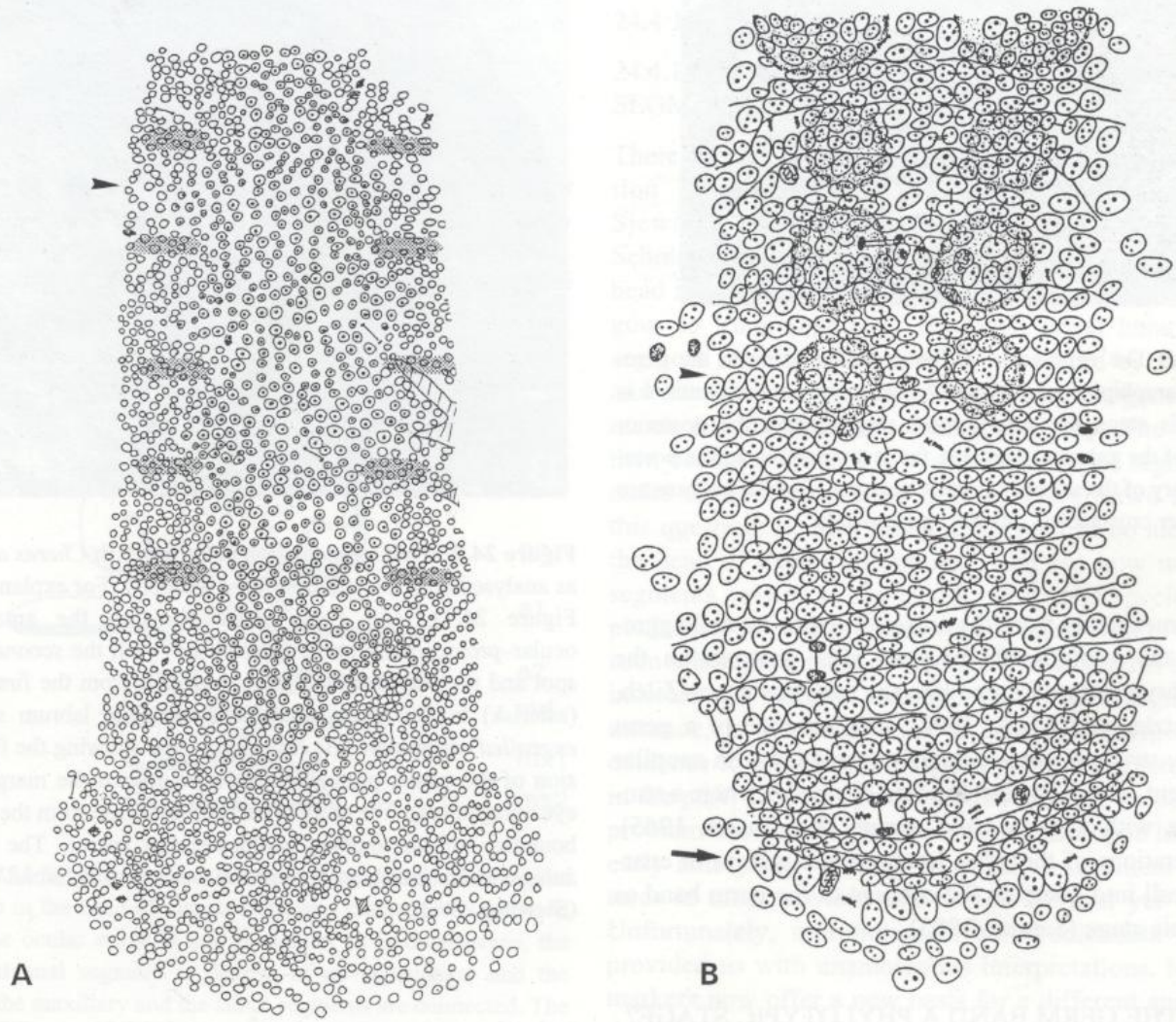

Figure 24.7 Posterior germ bands of an entomostracan crustacean *Leptodora kindtii* (A) (after Gerberding, 1997) and the malacostracan crustacean *Neomysis integer* (B) (after Scholtz, 1984). (A) *Leptodora* has no distinct growth zone; mitoses appear irregular and scattered throughout the germband. (B) *Neomysis* shows a teloblastic growth zone – large cells arranged in a transverse row with unequal divisions (arrow). Note the regular division pattern in the entire germband (for details, see Dohle and Scholtz, 1988). Arrowheads mark the position of the first thoracic segment.

Figure 24.8 The expression of the *engrailed* gene in the germ band of an amphipod crustacean (*Orchestia cavimana*) (anterior is up). At this stage the anteriormost stripe marks the posterior boundary of the antennal segment, the last stripe marks the posterior boundary of the seventh thoracic segment. Similar patterns are seen in other crustaceans, insects and annelids.

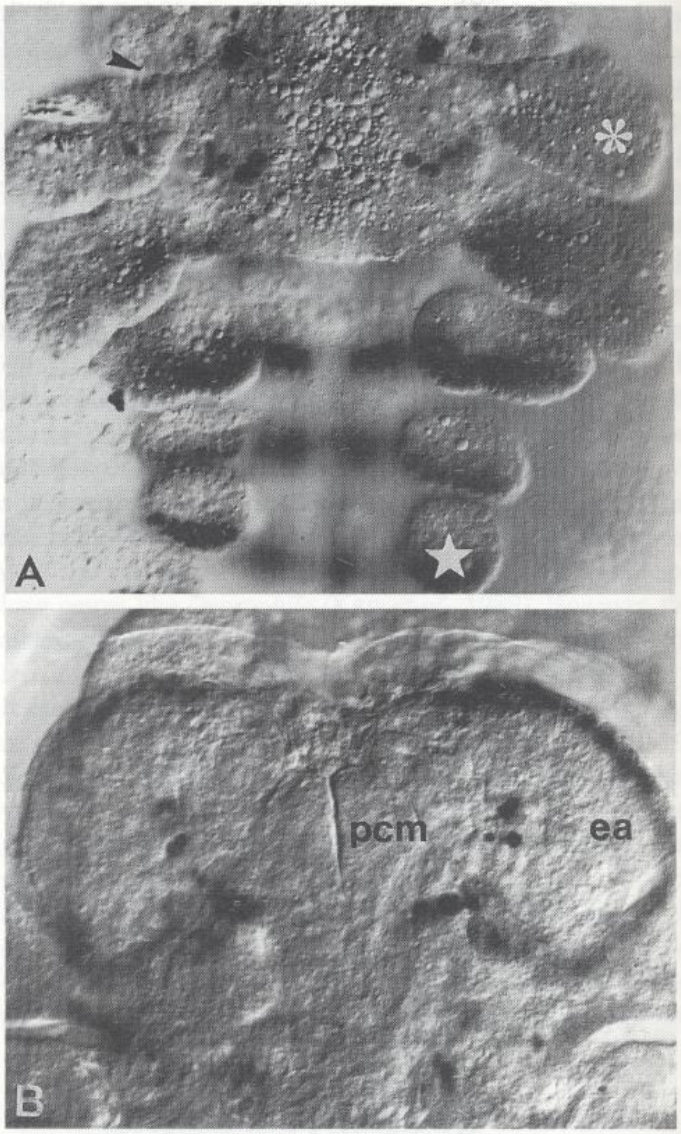

Figure 24.9 Head segmentation in the crayfish *Cherax destructor* as analysed with the anti-*engrailed* antibody. For explanation, see Figure 24.11. (A) Early stage showing the anteriormost ocular–protocerebral stripe (arrowhead) with the secondary head spot and the stripes marking the segments from the first antenna (asterisk) to the second maxilla (star). The labrum shows no *engrailed* expression. (B) Advanced stage showing the full extension of the ocular-protocerebral stripe around the margin of the eye anlagen (ea). The median parts of the stripe form the posterior boundary of the median protocerebrum (pcm). The ganglion anlage which has been thought to belong to a 'labral' segment (Siewing, 1963.)

is three-dimensional from the outset. There is no cell aggregation at the ventral side because after gastrulation the whole embryo is transformed into the nauplius larva (Zilch, 1978; Hertzler and Clark, 1992). The absence of a germ band *sensu stricto* is not necessarily correlated with naupliar development, as can be seen in some cirripedes where a nauplius larva with a germ band occurs (Kaufmann, 1965). These alterations of the germ band ground pattern in euarthropods call into question the concept of the germ band as a phylotypic stage (Sander, 1983).

24.3.3 IS THE GERM BAND A PHYLOTYPIC STAGE?

A phylotypic stage ('Körpergrundgestalt'; Seidel, 1960) is defined as the stage during ontogeny in which all representatives of a given animal group most resemble each other (Sander, 1983). The phylotypic stage is thought to represent a developmental constraint – a bottleneck – whereas earlier and subsequent stages show a much higher variability and freedom among the species of the group. It has been proposed that the germ band of arthropods is such a phylotypic stage (Sander, 1983). According to this view, the germ band would be the stage in arthropod development with the fewest differences between taxa and a necessary step in the

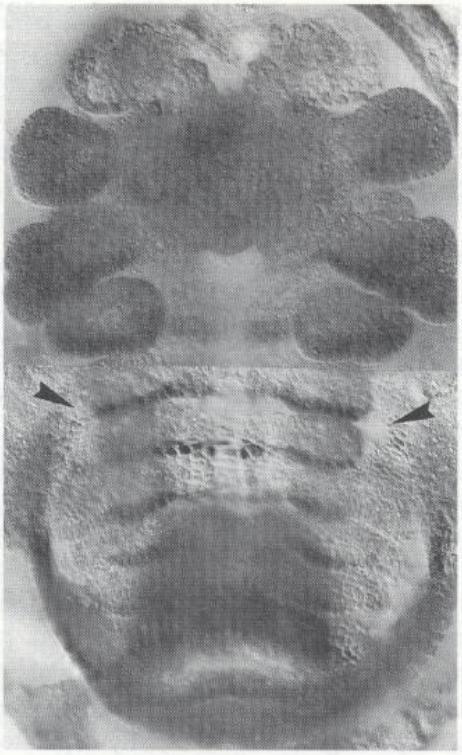

Figure 24.10 The expression of *engrailed* in the margin of the carapace of *Cherax destructor*. The stripe of the first maxillary segment continues into the circular carapace anlage (arrowheads). The stripes of the subsequent segments fuse also with the carapace margin.

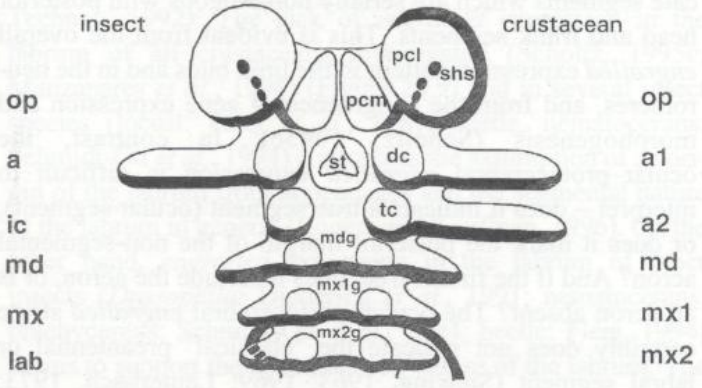

Figure 24.11 Schematic representation of the pattern of *engrailed* expression in the head of insects (left) and crustaceans (right). In insects, the ocular stripe is not as distinct as in crustaceans, the second antennal segment is devoid of an appendage, and the stripes of the maxillary and the labial segments are connected. The carapace stripe in the crustacean is also indicated. a, antenna; dc, deuterocerebrum; ic, intercalary segment; lab, labium; md, mandible; mdg, mandibular ganglion; mx, maxilla; mxg, maxillary ganglion; op, ocular–protocerebral region; pcl, lateral protocerebrum; pcm, median protocerebrum; shs, secondary head spot; st, stomodaeum; tc, tritocerebrum.

development of arthropods. Although it is clear that the arthropod stem species went through a germ band stage during embryonic development, this does not necessarily mean that this character is conserved throughout all recent arthropod species. In contrast, the concept of the phylotypic stage does not allow exceptions because of the constraints which stabilize this stage. It is well-documented that on each developmental level from the genes to organogenesis homologous structures can arise, despite alterations in preceding developmental stages (Sander, 1983; Dohle, 1989; Wagner and Misof, 1993; Scholtz and Dohle, 1996). Ontogenetic stages and processes can be altered without affecting the resulting product. There is no reason to believe that single developmental steps should be an exception to this rule, and the various modes of germ band formation and differentiation point in that direction.

24.4 HEAD SEGMENTATION

24.4.1 THE PROBLEMS OF ANALYSING HEAD SEGMENTATION

There are numerous theories concerning head segmentation in euarthropods (Weber, 1952; Manton, 1960; Siewing, 1963, 1969; Rempel, 1975; Bitsch, 1994; Scholtz, 1995c). There is no doubt that the euarthropod head is composed of segments which are serially homologous to trunk segments. However, serial homology and segment identity in the head are obscured by fusion, loss and alteration of structures, and by morphogenetic movements and displacement during ontogeny. The real issue then becomes the nature and the number of segments that make up the euarthropod head. There are two aspects to this question: (i) how many segments can be identified in the head of extant arthropods?; and (ii) how many head segments existed in the euarthropod stem species and its predecessors? The problem we are faced with is the definition of a segment and of the nature of structures that might indicate the former existence of a full segment. For instance, is the occurrence of coelomic cavities enough evidence to postulate the existence of a complete segment in the past? It seems plausible that the clue for solving the problem of segment number in the euarthropod head lies in early embryology. Here, the serial arrangement of structures is more obvious and fusion has not yet occurred. Unfortunately, morphological–embryonic data have not provided us with unambiguous interpretations. Molecular markers now offer a new basis for a different approach to the question of head segmentation. However, it is unlikely that the 'endless dispute' (Rempel, 1975) has come to an end or that the 'field of mental exercise' (Snodgrass, 1960) no longer exists.

Head segmentation involves two particular problematical issues:

1. The number and identity of segments in the anterior pregnathal area.
2. The posterior limit of the original euarthropod head.

In the anterior region, we face the problem of identification of putative segmental vestiges and of the serial homologization of segmental structures. Traditionally, these structures have been coelomic sacs, appendages, ganglia, sutures and muscles. In addition, we face the problem of whether arthropods possess an anteriormost non-segmental part, the acron. The existence of an acron has been deduced from the annelid prostomium which is formed by the larval episphere (Siewing, 1963) and which bears the eyes.

The problem of the posterior boundary of the arthropod head is of a different kind. It is related to the criteria that make up a head segment as opposed to a trunk segment. These criteria can be the extension of the head shield, the functional transformation of appendages into feeding or sensory appendages, the fusion of ganglia with anterior head ganglia, and differences in ontogenetic and genetic aspects of head versus trunk segment formation. For instance, malacostracan crustaceans show different cell division patterns in the naupliar and the post-naupliar regions (Dohle and Scholtz, 1988; Scholtz, 1990). The anteriormost segments up to the intercalary segment of *Drosophila* differ from more posterior segments in the mode of regulatory gene interactions (Cohen and Jürgens, 1991).

The possibility of using molecular markers in a variety of animals offers new ways to readdress questions of head segmentation. The expression pattern of the segment-polarity gene *engrailed*, for instance, has been used to analyse head segmentation patterns in various insects and crustaceans (Patel *et al.*, 1989; Schmidt-Ott and Technau, 1992; Fleig, 1994; Schmitt-Ott *et al.*, 1994; Scholtz, 1995c; Rogers and Kaufman, 1996). Unfortunately no data are available on chelicerates, myriapods, and representatives of onychophorans but some data exist on *engrailed* expression in the anterior region of annelids (Dorresteijn *et al.*, 1993; Lans *et al.*, 1993).

24.4.2 THE PREGNATHAL REGION

(a) The *engrailed* expression pattern as an analytical tool

engrailed provides us with a useful marker for embryonic segment anlagen before they are morphologically present. All *engrailed* stripes in the head region share several characteristic features which they also share with the more posterior trunk stripes (Scholtz *et al.*, 1994; Scholtz, 1995c): all are formed in a mediolateral progression, the distance between two adjacent stripes is the same, *engrailed* is expressed in cells on the surface first and later in neurogenic cells in the interior. In malacostracan crustaceans it has been shown that there are three distinct regions of *engrailed* expression in the pregnathal area (Scholtz, 1995c). These are in anteroposterior sequence, the ocular–protocerebral stripe, the first antennal/deuterocerebral stripe, and the second antennal/tritocerebral stripe (Figures 24.9 and 24.11). A corresponding pattern has been described for several insects such as beetles (Fleig, 1994; Brown *et al.*, 1994; Schmidt-Ott *et al.*, 1994), locusts (Patel *et al.*, 1989), various dipterans (Schmidt-Ott *et al.*, 1994) and others (Rogers and Kaufman, 1996). The pattern in *Drosophila melanogaster* is somewhat more complicated and the various *engrailed* stripes and patches have been interpreted differently with regard to segment sequence, number and identity (Diederich *et al.*, 1991; Schmidt-Ott and Technau, 1992; Jürgens and Hartenstein, 1993). However, a recent study on the metameric subdivision of the anterior *Drosophila* brain using confocal laser scanning microscopy revealed an *engrailed* expression pattern identical to that of crustaceans and other insects (three pregnathal stripes) (Hirth *et al.*, 1995). The similarity between insects and crustaceans extends even to characteristics of individual stripes. For instance, the stripes of the ocular–protocerebral region and the first antennal segment are medially separated (Figures 24.9 and 24.11). Furthermore, a secondary head spot occurs in the eye region which has been described in *Drosophila*, the beetle *Tribolium* and crustaceans (Schmidt-Ott and Technau, 1992; Brown *et al.*, 1994; Scholtz, 1995c) (Figures 24.9 and 24.11).

(b) The nature of metameres

The *engrailed* stripes in the first antennal/deuterocerebral and the second antennal/tritocerebral regions clearly indicate segments which are serially homologous with posterior head and trunk segments. This is evident from the overall *engrailed* expression pattern in the limb buds and in the neuromeres, and from the congruence of gene expression and morphogenesis (Scholtz, 1995c). In contrast, the ocular–protocerebral *engrailed* expression is difficult to interpret – does it indicate a true segment (ocular segment), or does it mark the posterior margin of the non-segmental acron? And if the first is true, does it include the acron, or is an acron absent? The ocular–protocerebral *engrailed* stripe certainly does not indicate the 'classical' preantennal or labral segment (Siewing, 1963, 1969; Lauterbach, 1973; Jürgens and Hartenstein, 1993) which has been thought to lie between the eye-bearing acron and the (first) antennal segment. This is because the ocular–protocerebral stripe comprises the eye anlagen, which have been thought to indicate the acron together with the brain parts attributed to the labral segment (Siewing, 1963) (Figs 9, 11). This continuous stripe shows that these structures form **one** unit and not two subsequent ones. Furthermore a mutant analysis in *Drosophila* shows that there is no evidence for a labral segment situated between the ocular region and the antennal segment (Schmidt-Ott *et al.*, 1995). If the ocular–protocere-

bral *engrailed* stripe does indicate a segment, it would be an ocular segment which has been postulated by several authors (Reichenbach, 1886; Sharov, 1966; Schmidt-Ott and Technau, 1992; Schmidt-Ott *et al.*, 1995; Rogers and Kaufman, 1996). The acron would then either be included in this segment or absent. However, there are arguments in favour of the acron nature of the body part marked by the ocular–protocerebral *engrailed* stripe. The posterior margin of the episphere of the trochophora larva of the polychaete *Platynereis* seems to express *engrailed* (Dorresteijn *et al.*, 1993). Since this is the anteriormost *engrailed* stripe in the polychaete it might correspond to the anteriormost stripe in arthropods. The leech homologue of the *Deformed* gene of *Drosophila*, *Lox 6*, is expressed in the third suboesophageal ganglion of *Hirudo medicinalis* (Aisemberg *et al.*, 1995). In insects, *Deformed* is expressed in the mandibular and maxillary segments (Diederich *et al.*, 1991; Fleig *et al.*, 1992). Thus, the supraoesophageal ganglion of annelids would correspond to the protocerebrum of arthropods and again the first *engrailed* stripe would mark the acron in arthropods (insects and crustaceans).

(c) The nature of the labrum

The labrum originating from a bilobed anlage is an apomorphy of the euarthropods. A corresponding structure occurs in neither onychophorans nor annelids. The labrum has been interpreted as being a ventral outgrowth of the acron anterior to the mouth (Walossek and Müller, 1990), as being the medially fused appendages of a preantennal (labral) segment (Siewing, 1969; Lauterbach, 1973; Weygoldt, 1979) or as the anteriormost body segment (Sharov, 1966; Schmidt-Ott and Technau, 1992). The lack of *engrailed* expression in the labrum of all crustaceans investigated (Scholtz, 1995c; Manzanares *et al.*, 1996) (Figure 24.9) and in several insect species (locust: Patel *et al.*, 1989; beetle, nematocerans: Schmidt-Ott *et al.*, 1994) contradicts the assumption of an origin of the labrum from appendages and the segmental nature of the labrum in general (Rogers and Kaufman, 1996). On the other hand, *engrailed* expression in the labrum of other insects (*Drosophila*: Diederich *et al.*, 1991; nematocerans, brachycerans: Schmidt-Ott *et al.*, 1994; beetle: Fleig, 1994) seems to support the appendiculate nature of the labrum. The *Distal-less* gene is expressed in the tips of limb buds in representatives of all higher euarthropod groups (Panganiban *et al.*, 1995). The *Distal-less* expression in the labrum of some crustaceans and insects (Panganiban *et al.*, 1995) seems to argue even more strongly for a possible homology between the labrum and segmental appendages. However, if the labrum is considered as being leg-like in its nature the question arises as to which segment it belongs. In addition, from the distribution of the labral *engrailed* expression amongst insects it has been concluded that this is a secondary feature in some insects (Scholtz, 1995c). Morever, the correlation between *engrailed* expression and morphogenesis is different between legs and the labrum – in the labrum *engrailed* expression does not precede morphogenesis as is the case in limb bud areas, the labrum is a morphologically well defined structure before *engrailed* expression occurs (see Scholtz, 1995c). This makes the homology between the labrum and legs doubtful (see Dickinson 1995; Bolker and Raff, 1996). The interpretation of the pattern of *Distal-less* expression is also problematic. Although it is clearly expressed in the tips of limbs of insects, myriapods, chelicerates and crustaceans it is also expressed in the telson of crustaceans (Panganiban *et al.*, 1995). The telson is clearly no limb derivative. Claims that *Distal-less* expression is indicative of a limb character of the labrum are, therefore, doubtful. Also since *Distal-less* expression is not restricted to tips of limb buds it seems likely that the *Distal-less* expression in the labrum is comparable with that in the telson and that labrum and telson mark the extreme ends of the body axis.

The evidence for the labrum being the fused appendages of a preantennal segment situated between the acron and the (first) antennal segment appears weak. It is more likely that the labrum is the tip of the acron which moved ventrally and posteriorly during evolution of the euarthropods.

24.4.3 THE POSTERIOR BOUNDARY OF THE HEAD

engrailed is expressed in the gnathal region of crustaceans and insects in the segments of the mandibles, (first) maxillae and second maxillae (labium). There is no trace of an additional segment between the second antennal (intercalary) segment and the mandibular segment such as postulated by Chaudonneret (1987) (Figures 24.9 and 24.10). There is also no distinct separation from the trunk segments. Nevertheless, the pattern of *engrailed* expression might contribute to the recognition of the original posterior boundary of the euarthropod head. In embryos of the decapod crustacean *Cherax destructor* the cells of the margin of the developing carapace express *engrailed* (Figure 24.10). This *engrailed* expression continues into the *engrailed* stripe of the first maxillary segment (Figure 24.10). It is concluded that the carapace margin is the extended posterior margin of the segment of the first maxillae. The *engrailed* stripes of the subsequent second maxillary segment and the trunk segments fuse laterally with this circular *engrailed* region (Figure 24.10). These findings speak against an origin of the carapace from the second maxillary segment (Lauterbach, 1974; Newman and Knight, 1984) or the antennal segment (Casanova, 1993) – at least ontogenetically. Moreover, the results presented here might point towards the ground pattern of the euarthropod head. In several insects, the *engrailed* stripes of the maxillary and the labial segments are connected by a bow-like region of *engrailed* positive cells (Patel *et al.*, 1989; Diederich *et al.*, 1991; Fleig *et al.*, 1992; Brown *et al.*, 1994; Rogers and Kaufman, 1996) (Figure 24.11). These comparable expression patterns found in the crustacean *Cherax* and in insects

can be interpreted as indicating the original posterior margin of their heads. This supports the idea that in the stem species of the mandibulates only the first maxillary segment was included in the head (Lauterbach, 1980; Walossek and Müller, 1990), a hypothesis based on the fact that the cephalocarid crustaceans possess only one pair of maxillae. The 'second maxillae' do not differ from the thoracic appendages (Sanders, 1963). The pattern of maxillary muscle attachment in other crustaceans (Pilgrim, 1973) and the embryonic development of the head shield in centipedes (Dunger, 1993) also support this hypothesis. Furthermore, trilobites show a head shield that covers the antennal segment and three postantennal segments (Cisne, 1975; Walossek, 1993) (Figure 24.12). The posterior head margin in embryonic insects and crustaceans as indicated by the (first) maxillary *engrailed* expression would correspond, as a recapitulation, to the posterior margin of the trilobite head as indicated by the head shield.

24.4.4 THE GROUND PATTERN OF THE EUARTHROPOD HEAD

To summarize, the original euarthropod head probably comprised an acron, an antennal segment and three postantennal segments, all covered by a head shield. This is at least true for the crown-euarthropods (the descendants of last common ancestor of extant euarthropods; see Jefferies, 1980). In the stem lineage of euarthropods there are certainly representatives with fewer cephalic segments (e.g. *Sidneyia*; Bruton, 1981). The acron bears the compound eyes, the protocerebrum and the labrum. The deuterocerebrum is the ganglion of the antennal segment. The tritocerebrum is the ganglion of the first postantennal segment. Originally, it lay posterior to the mouth as can be seen in crustacean and insect embryos (Weygoldt, 1979; Boyan *et al.*, 1995) and some adult crustaceans such as branchiopods (Hanström, 1928). The subdivision of the head into pregnathal and gnathal areas is an apomorphy of the mandibulates. Within the mandibulates the 'second maxillary (labial in insects) segment' became fused to the head and its appendages altered their function into head limbs in different lineages independently. Insects and myriapods lost the appendages of the original first postantennal segment, but retained the tritocerebrum and the corresponding *engrailed* stripe (unknown for myriapods). As mentioned above, there are no *engrailed* data available for chelicerates. The general opinion is that chelicerates reduced the antennal segment (Weygoldt, 1985). However, most embryonic studies in chelicerates have been biased by the idea that there is a preantennal (labral) segment (Pross, 1966; Winter, 1980; Weygoldt, 1985). This led to some confusions concerning the coelomic cavities and ganglion anlagen (Pross, 1977; Weygoldt, 1985). Within the concept of euarthropod head segmentation discussed here, the characteristics of chelicerate embryos can be more easily interpreted. One has to look for vestiges of only one precheliceral segment. Nevertheless, a study using molecular markers to analyse the segmentation pattern in chelicerates is badly needed.

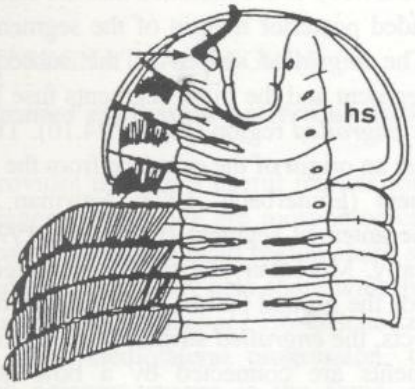

Figure 24.12 The head region of a trilobite (after Brusca and Brusca, 1990, from Cisne, 1975). The head shield (hs) covers the antennal segment (arrow) and three postantennal segments.

24.5 SOME APOMORPHIES SUPPORTING ARTHROPOD AND EUARTHROPOD MONOPHYLY

CLEAVAGE

1. The radial position of the cleavage products is an apomorphy for arthropods. Plesiomorphically there was spiral cleavage.
2. The superficial cleavage type or mixed cleavage type might be an arthropod apomorphy. Comparable developments do not occur in annelids.
3. The blastoderm stage with a central yolk mass is an arthropod apomorphy. In spiral cleavage there is originally a coeloblastula.

GERM BAND

1. The formation of a germ band by aggregation of blastomeres on the ventral side of the germ is an apomorphy of arthropods. Polychaetes have originally no germ bands [exceptions can be found in species with a large amount of yolk (A. Dorresteijn, personal communication)]. The whole germ is transformed into the trochophora larva and the later worm. Clitellates possess germ bands. These are, however, formed on the dorsal side of the germ and migrate and fuse ventrally (Penners, 1924; Smith *et al.*, 1996). This germ band type is a clitellate apomorphy.
2. The head lobes are an arthropod apomorphy since they are also found in onychophorans (Anderson, 1973; Walker, 1995). There is no corresponding structure in annelids.
3. A non-teloblastic growth zone is an arthropod apomorphy, whereas in annelids the mesoderm buds from mesoteloblasts which are derivatives of the 4d cell.

HEAD SEGMENTATION

1. A labrum originating from a bilobed anlage is an apomorphy of euarthropods. A corresponding structure can be found neither in onychophorans nor in annelids.
2. A head consisting of an antennal and three postantennal segments is an apomorphy of crown-group euarthropods. The onychophoran head is difficult to interpret but it probably consists of the eye bearing acron, the antennal segment, the jaw segment, and the segment of the oral papilla.

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