

Clonal analysis of the crustacean segment: the discordance between genealogical and segmental borders

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Summary

The post-naupliar germ bands of many higher crustaceans show a regular grid-like pattern of cells. This pattern is generated, in part but not *in toto*, by the proliferation of teloblasts.

The exact lineage of all the cells of the post-naupliar germ band has been investigated in most of the orders belonging to the monophyletic unit Peracarida (Cumacea, Tanaidacea, Isopoda, Mysidacea, Amphipoda). The cell divisions and differentiation could be followed up to the formation of appendage buds, of ganglia and of intersegmental furrows.

The most-striking result is that the genealogical borders between cells of different clones do not correspond to the transverse intersegmental furrows. Instead, the genealogical borders divide a segment, and even the appendage buds, into anterior and posterior

compartments.

There are different pathways for the formation of the cells of the post-naupliar germ band, though the subsequent differentiation may be nearly identical. It has been deduced from these findings that the fate of the cells is not determined by their origin. This supposition could be substantiated by a comparative analysis of the different orders. In Amphipoda, for instance, ectoteloblasts are not differentiated; the post-naupliar germ band is formed by an assemblage of blastoderm cells. Nevertheless, the cleavage pattern of these cells is for the most part identical to that of the other orders that possess ectoteloblasts.

Key words: clonal analysis, segmentation, Crustacea, cell lineage, germ band.

Introduction

The analysis of mutants is an important tool for the elucidation of factors and genes controlling segmentation. Up to the present time, this analysis has been restricted to *Drosophila* (e.g. Lewis, 1978; Nüsslein-Volhard & Wieschaus, 1980; Akam, 1987).

However, other approaches can also be applied to the analysis of factors responsible for the differentiation of segmentally repeated structures. By microsurgical treatment, material can be transplanted or deleted in order to test the degree of autonomy and commitment, and the role of induction or regulation (e.g. Seidel *et al.* 1940; Sander, 1960; Doe & Goodman, 1985*a,b*; Technau, 1987; Penners, 1934, 1937; Shankland & Weisblat, 1984; Shankland, 1987*a,b*).

Within the vast bulk of animals where experimental manipulation is difficult or impossible, a more formal analysis of different 'instructions for differentiation' is feasible by a *comparative approach*. By comparing

closely related species, one can find slight differences in morphogenetic events, which reflect the stepwise phylogenetic alterations. They are, in many cases, even more subtle than those found in mutant embryos of the same species. One takes, so to speak, the 'mutants' provided by the evolutionary process in order to work out and interpret the morphogenetic differences.

The differentiation of structures during morphogenesis is always a highly coordinated process. Especially in development with strict cell lineages, one division or one differentiation follows nearly inevitably after the other, and the epigenetic events seem to roll on without much interference by independent factors. The contribution of a comparative approach to the understanding of the differentiation process might be to analyse which steps are not necessarily coupled with, or dependent on, the preceding steps. The result of a comparative analysis can be the conclusion that one division or one differen-

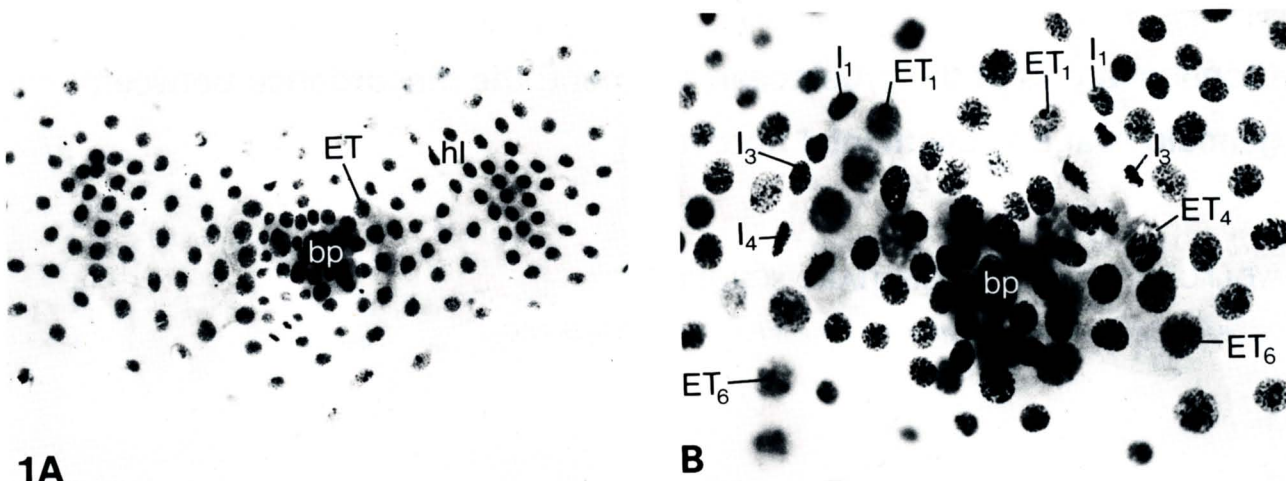


Fig. 1. Ectoteloblast differentiation in *Diastylis*. (A) The ectoteloblast precursors (ET), which first differentiate caudal to the blastopore (bp), migrate around the blastopore on both sides. Cells are concentrated at the presumptive head lobes (hl). Anterior is up in this and all subsequent figures except Figs 8, 10, 11 and 14A. (B) The ectoteloblasts (ET) bud off the first descendants. On the animal's right side, four ectoteloblasts have each budded off a small descendant cell (I₁–I₄). On the left side, one descendant cell has been budded off (I₁), ET₂ and ET₃ are in anaphase, ET₄ is in prophase.

tiation is *not* an inevitable prerequisite for the subsequent divisions or differentiations. Of course, we cannot say anything about the material nature of the factors; however, we can say something about the *independence* of one step from the other. Comparative embryological analyses can result in the 'uncoupling' of factors or events that seem to be closely coordinated or correlated with each other in normal development.

An example of this approach is the analysis of segmental structures in the germ band of the Peracarida, malacostracan crustaceans which possess a brood pouch. Members of the Cumacea (Dohle, 1970, 1976a), Tanaidacea (Dohle, 1972), Isopoda (Hahnenkamp, 1974), Mysidacea (Scholtz, 1984) and Amphipoda (Scholtz, 1986) have been analysed from the first appearance of teloblast precursors and definable blastoderm cells up to the formation of intersegmental furrows, limb buds and ganglion rudiments.

Segment formation in the cumacean *Diastylis*

It was first established in the cumacean *Diastylis rathkei* that there is an invariable cleavage pattern of cells in the post-naupliar segments of Peracarida (Dohle, 1970, 1976a). A brief description of morphogenetic events as a basis for a comparative discussion will be useful. The early divisions are superficial; they take place without cytokinesis. The first obvious differentiations can be detected after the migration of the nuclei into the periplasm. Cells concentrate in an area around the blastopore where germ cells and mesentoderm cells migrate into the yolk. Caudal to

this area the precursor cells of ectoteloblasts can be distinguished as a crescent of cells with large nuclei. These cells migrate around the blastopore on both sides (Fig. 1A) and meet in front to form a crescentic row. These ectoteloblast cells bud off small cells with darkly staining nuclei anteriorly (Fig. 1B). There is a mitotic wave starting from anteromedian ectoteloblasts and progressing posterolaterally. Further small cells are budded off successively. The small cells are arranged in longitudinal and transverse rows so that a beautiful grid-like pattern is formed.

It can be demonstrated that in front of the first transverse cell row of ectoteloblastic origin, several rows are formed by cells that had previously been scattered on the germ disc (Fig. 2A). These cells must have been forced into the pattern by the influence of a 'row-forming factor'. These cells of non-ectoteloblastic origin will produce the ectodermal material for the first and second maxillary segments and also for the anterior part of the first thoracic segment. Rows of ectoteloblastic origin are designated by latin numbers (row I, II, III, etc.), the rows of non-ectoteloblastic origin are designated by arabic numbers in brackets (row (0), (1), (2), (3)). The distance of the cells from the midline is designated by index numbers; the cell nearest to the midline is named 1 (e.g. I₁), the next cell 2 (e.g. I₂), etc. The cells of all rows except (0) and (1) cleave twice by a mediolateral mitotic wave to give rise to four rows of cells, named a, b, c and d. Then the cells pass into differential cleavages; each cell divides in a characteristic and recognizable manner (Fig. 2B). The cleavages are stereotyped and invariant with regard to the

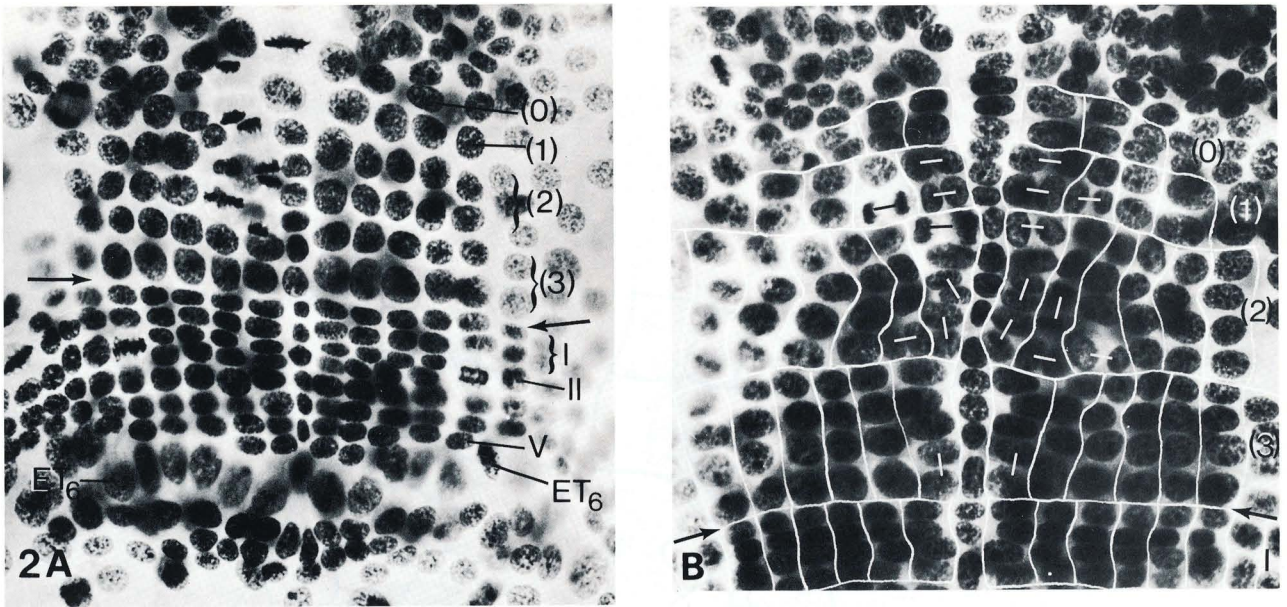


Fig. 2. Post-naupliar germ bands in *Diastylis*. (A) A stage in which two cells of the sixth row and five cells of the fifth row of ectoteloblastic derivatives (V) have been given off by the ectoteloblasts (ET) on each side. On the right side, ET₆ is in telophase, on the left side, ET₆ is in prophase. The cells of the first two rows of ectoteloblastic origin (I and II) have already been divided once by a mediolateral mitotic wave. In front of row I, four rows (rows (0), (1), (2) and (3)) have been formed by cells of the blastodermic germ disc. Of these, rows (2) and (3) have already been divided once by a mitotic wave to form two rows each. On the animal's right side, the two cells nearest to the median line cleave for the second time and two cells of row (1) cleave for the first time. The border between cells of non-ectoteloblastic and of ectoteloblastic origin is indicated by arrows. (B) Detail of a stage in which the eighth row of ectoteloblastic derivatives is generated. Progeny of rows (0), (1), (2), (3) and I are shown. Cells of one clone are surrounded by white lines. Nuclei after the first differential cleavage are connected by straight lines. The border between cells of non-ectoteloblastic and of ectoteloblastic origin is indicated by arrows.

direction of the spindle and the size and position of the daughter cells. There is only slight variation in the sequence of cleavages. It is possible to establish unequivocally the lineage of the cells on the post-naupliar germ band by the characteristics: orientation, inequality and timing of divisions. Analyses of early stages are shown in Figs 2B and 3.

The most remarkable results of the analyses are as follows. The descendants of the cells in rows (2) and (3), which are of non-ectoteloblastic origin, show differential cleavages that are nearly identical to the cleavages of corresponding cells in rows of ectoteloblastic origin (Fig. 4). However, there are slight differences in some cleavages. These differences are pointed out by arrows in Fig. 4. The cell (2)a₂ divides like (3)a₂ and unlike Ia₂. This could suggest that the cells in the non-ectoteloblastic rows differ slightly from those of ectoteloblastic origin. In contrast, the cell (3)d₃ divides like Id₃ and unlike (2)d₃. How cells divide can vary regardless of their origin and without affecting the surrounding cell pattern.

The limb buds can first clearly be seen at the stage depicted in Fig. 3. Further cleavages of the cells can be traced to a stage shown in Fig. 6. An appendage bud is composed of cells contributed by different cell

clones. The anterior part of an appendage bud is made up of the posterior descendants of a cell row, the posterior part of the same appendage bud is made up of the anterior descendants of the subsequent cell row. The genealogical border between two rows runs transversely across the limb bud. In other words, anterior cells of one cell clone contribute to the hind part of an anterior limb; posterior cells of the same cell clone contribute to the front part of the following limb (Fig. 5). The genealogy of the cells constituting the first and second maxillae and the first and second thoracic limbs are represented schematically in Fig. 7. It may be noted that the genealogical border between cells of non-ectoteloblastic origin (cells of row (3)) and cells of ectoteloblastic origin (cells of row I) divides the first thoracic segment.

The ganglion rudiment is a composite structure, too. Descendants of the cells c₁, c₂, d₁ and d₂ contribute to the formation of neuroblasts, but descendants of the cell a₁ of the following row also take part in the formation of a ganglion rudiment. The investigation of the exact genealogy of the neuroblasts reveals that not all cells of a clone become neuroblasts (Fig. 8). Cell a₁ divides into an inner cell a₁i and an outer cell a₁e. Cell a₁i generates two

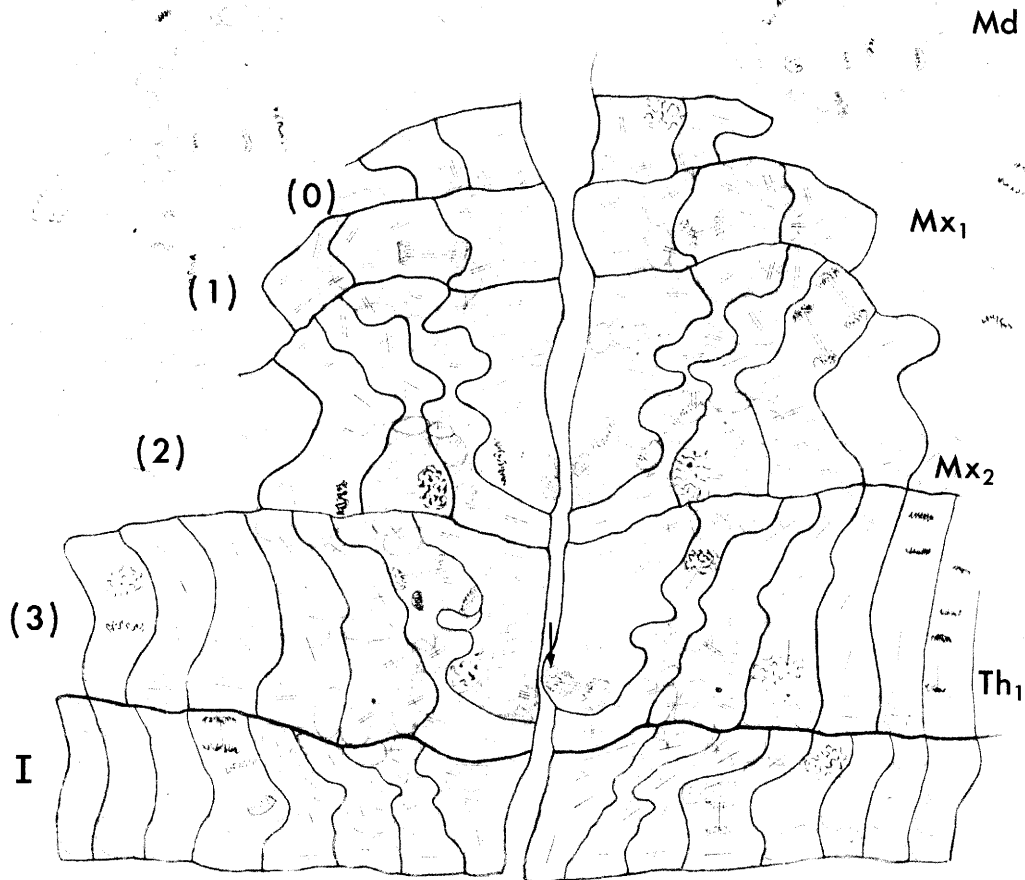


Fig. 3. Cell clones on the germ band of *Diastylis*. Detail of a germ band including the rudiments of the mandibles (Md), the first maxillae (Mx₁), the second maxillae (Mx₂), and the first thoracic limbs (Th₁). The descendants of the cells of the ectodermal rows (0), (1), (2), (3) and I are shown. Cells which have originated from one cell are surrounded by a thick line. Nuclei of sister-cells are connected by thin straight lines. One line denotes the first differential cleavage, two lines denote the second, and three lines denote the third differential cleavage. Bulging limb buds are shaded. Small ectodermal nuclei are grey; the first ganglion mother cell nuclei are dark grey. In row (3) the second ganglion mother cell has been budded off (marked by an arrow).

neuroblasts, whereas a₁e gives rise to epidermal cells. The neuroblasts bud off small ganglion mother cells into the interior of the embryo. After the generation of a ganglion mother cell the larger cell is not definitely determined as a neuroblast. Cell d₁h generates two ganglion mother cells, d₁hg and d₁hng. The neuroblast d₁hnn then divides on the surface of the egg into two large cells, d₁hnni and d₁hnne. The inner cell, d₁hnni, gives off a ganglion mother cell and becomes a neuroblast, d₁hnnin; the outer cell, d₁hnne, divides into two epidermal cells, d₁hnnei and d₁hnnee. The first neuroblasts and ganglion mother cells are shown in Fig. 9. The ganglion mother cells

are arranged in columns. They divide once, giving rise to ganglion cells (Fig. 10).

The intersegmental furrow does not mark any genealogical border. On the contrary, it runs transversely and slightly obliquely through the descendants of one row (Fig. 6). It passes behind descendants of b₁, through descendants of b₂ and b₃, then moves in front of b₄ and b₅ and passes through a₆ and a₇. Though the furrow is always formed between certain cells, it is not determined by their genealogy.

The mesoderm of the post-naupliar germ band can be traced back to two pairs of mesoteloblast mother cells. Each mother cell delivers one cell which mi-

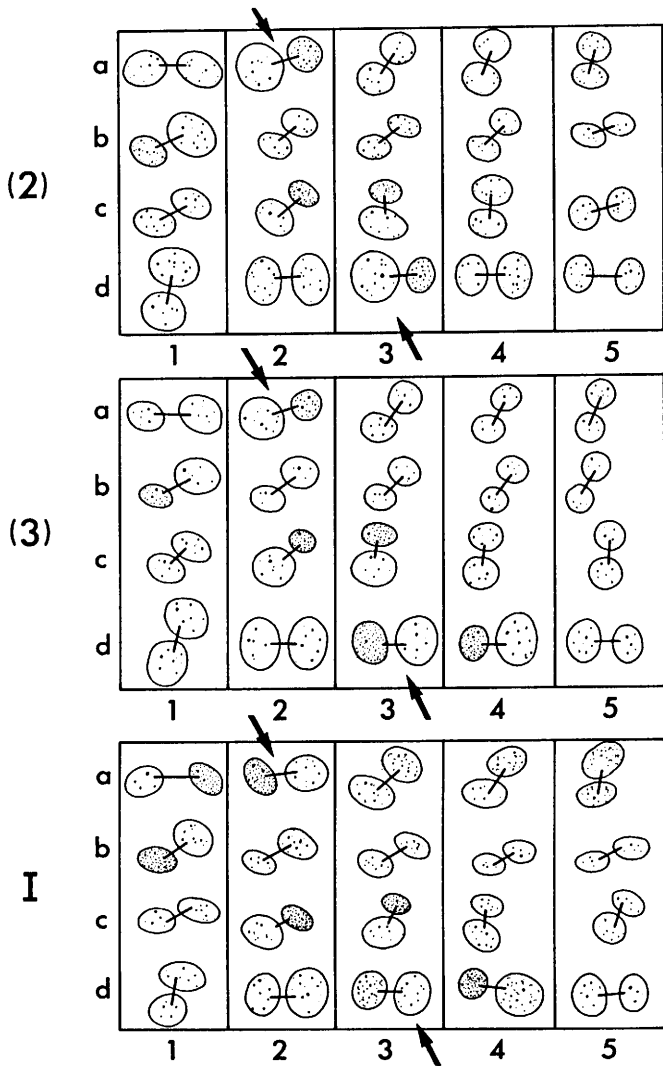


Fig. 4. The first differential cleavages in *Diastylis*. The first differential cleavages of the cells forming the ectodermal rows (2) and (3), which are of blastodermic origin, and of row I, which is of ectoteloblastic origin, are shown schematically. The animal's left side is shown. Differences between rows are marked by arrows.

grates beneath the ectodermal cell row (2). After a complicated division pattern which is shown in Fig. 11, four pairs of mesoteloblasts are generated. Under the ectodermal cell row (3) only three pairs of mesoderm cells can be found (Fig. 11). Row I has the full complement of four pairs of mesoderm cells (Fig. 16). A final row or ring of four pairs of mesoteloblasts is characteristic of all Malacostraca.

Comparison with other Peracarida

In the same way as *Diastylis*, several other peracarid crustaceans have been analysed in order to find similarities and divergent characteristics. Only the most striking differences are summarized.

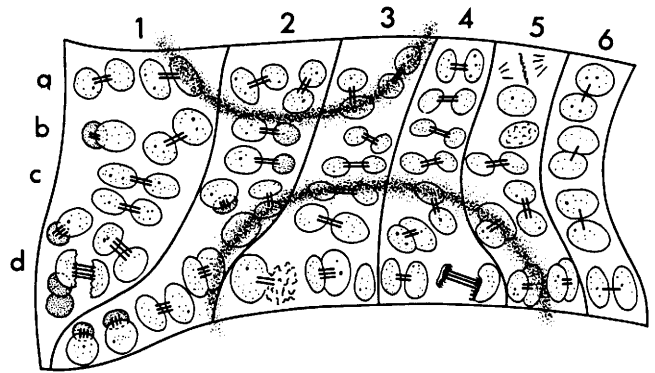


Fig. 5. Descendants of six cells of row (3) in *Diastylis*. The animal's left side is shown. Cells that have originated from one cell are surrounded by thick lines. Nuclei of sister cells are connected by thin straight lines. One line denotes the first, two lines denote the second differential cleavage etc. Six ganglion mother cells have been budded off to the interior of the embryo. The posterior part of the second maxilla and the anterior part of the first thoracic limb are shaded.

Tanaidacea

In the tanaidacean *Leptochelia*, there is no migration of ectoteloblast precursors around the blastopore (Dohle, 1972). The ectoteloblasts are differentiated *in situ*. The first row of ectoteloblastic derivatives is much more difficult to identify, as these cells are not budded off in a mediolateral wave. Row III and the subsequent ones are budded off as in *Diastylis*. In later germ bands, the limb buds of the second thoracic segment are further differentiated than the limb buds of the first thoracic segment and of the second maxilla. This is caused by the fact that the row in front of row I cleaves three times so that eight rows are generated. These are homologous to the two sets of four rows generated by the rows (2) and (3) in *Diastylis*.

Isopoda

In the isopods *Asellus aquaticus* and *Ligia oceanica*, the formation of the post-naupliar germ band is basically like that in *Diastylis* (Hahnenkamp, 1974). There are slight deviations of which only one will be mentioned. As in *Diastylis*, the cells of the two rows (2) and (3) of blastodermic origin and the cells of the rows of ectoteloblastic origin cleave twice, resulting in the formation of four rows, a, b, c and d. In *Diastylis* and in other peracaridans, the wave of differential cleavages begins in row d, followed by row c and row a, the cells of row b are lagging behind. In isopods, it is, on the contrary, row b which is the first to divide. This has not the slightest effect on the pattern of subsequent cleavages. The cells in isopods cleave in a way identical to cumaceans or amphipods.

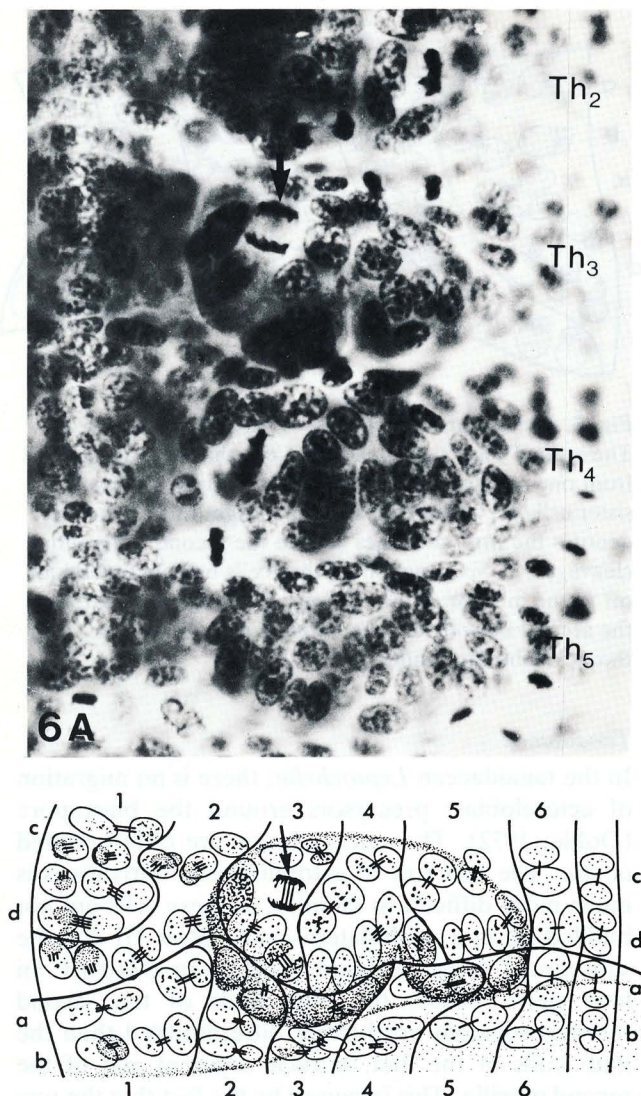


Fig. 6. Differentiation of the thoracic limbs in *Diastylis*. (A) Detail of the left side of a post-naupliar germ band with rudiments from the second to the fifth thoracic limb (Th₂ to Th₅). (B) Clonal analysis of the third thoracic limb bud from the same preparation (compare the anaphase figure of cell IIc₃hh, marked by an arrow). Only the descendants of IIc and d and of IIIa and b are shown. The limb bud is composed of cells originating from IIc₃₋₅, IId₃₋₅, and IIIa₂₋₅. The intersegmental furrow is drawn as a shaded line. It passes obliquely from posterior of descendants of IIIb to descendants of IIIa.

The d row is not determined to form the centre of the ganglion or the apex of an appendage bud by being first to start differential cleavage.

Mysidacea

While, in Cumacea, Tanaidacea and Isopoda, the germ band is stretched out on the egg surface, in Mysidacea, a caudal papilla is formed. This does not affect the differentiation of rows and segments in the

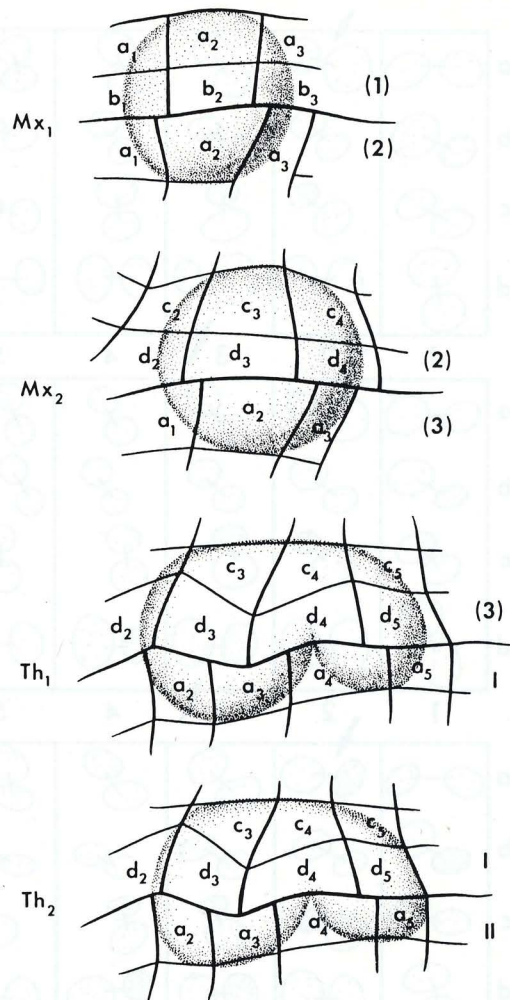


Fig. 7. Schematic representation of the composition of appendage buds by different cell clones in *Diastylis*. The first maxilla (Mx₁), second maxilla (Mx₂), first thoracic limb (Th₁) and second thoracic limb (Th₂) are analysed.

post-naupliar region which is similar to the foregoing orders (Scholtz, 1984). The most obvious difference is the highly differentiated naupliar region, especially the first and second antennae, when compared with the post-naupliar region and equivalent stages of other Peracarida (Fig. 12).

Amphipoda

From the observation that cells of different origin show the same differential cleavage pattern, it has been deduced that the generation of cells from ectoteloblasts is not a prerequisite for a particular differentiation. In principle, the same cleavage patterns could be realized without ectoteloblast formation. An experimental tool to test this assumption would be the ablation of the ectoteloblast precursors. This has not proved to be feasible. However, the evolutionary process has performed an equivalent experiment. In amphipods no differentiation of ecto-

teloblasts takes place (Dohle, 1976*b*; Scholtz, 1986). All the cells of the post-naupliar germ band are cells developed from the germ disc. The blastodermic cells, which are scattered at first, are forced into longitudinal and transverse rows (Fig. 13). These divide in the same manner as cells of ectoteloblastic origin in other species. Because of their special characteristics, it is easy to identify the descendants of row (4) as homologues to the descendants of row I in other Peracarida. The genealogical border between

cells of two rows again runs transversely through the appendage bud (Fig. 14).

It must be stressed that the mode of formation of the whole germ band out of scattered blastodermic cells in amphipods is clearly derived phylogenetically from the formation of the posterior part of the germ band by ectoteloblasts. All Malacostraca except the amphipods possess ectoteloblasts. The specific pattern of differential cleavages is an acquisition of the ancestor species of the Peracarida, and has 'survived'

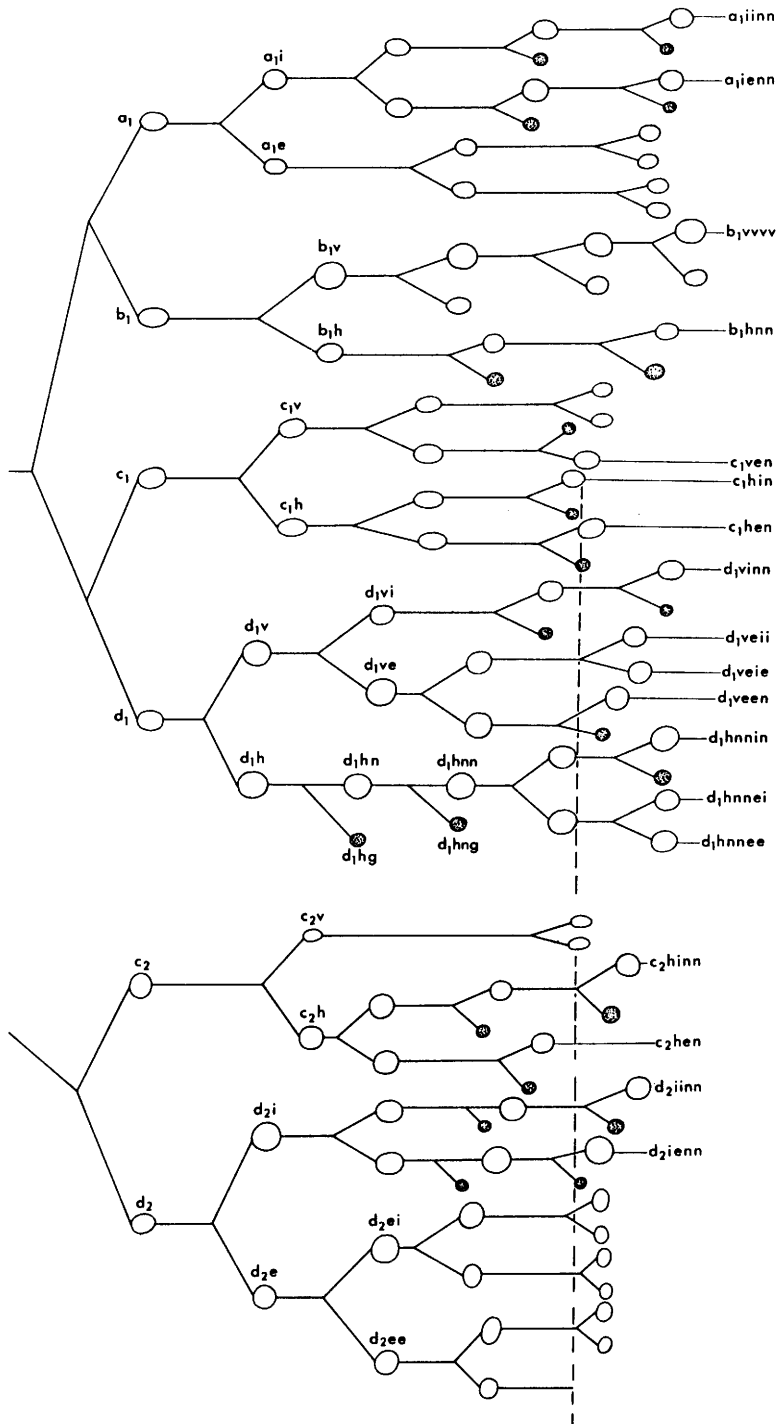


Fig. 8. Cell lineage of the ectodermal cells IIa₁–II d₁ and IIc₂–II d₂. Ganglion mother cell nuclei are shown as small circles with dark shading. They are designated by the final letter g. Neuroblasts are designated by the letter n. The stage that is shown in Fig. 9 is indicated by the broken line.

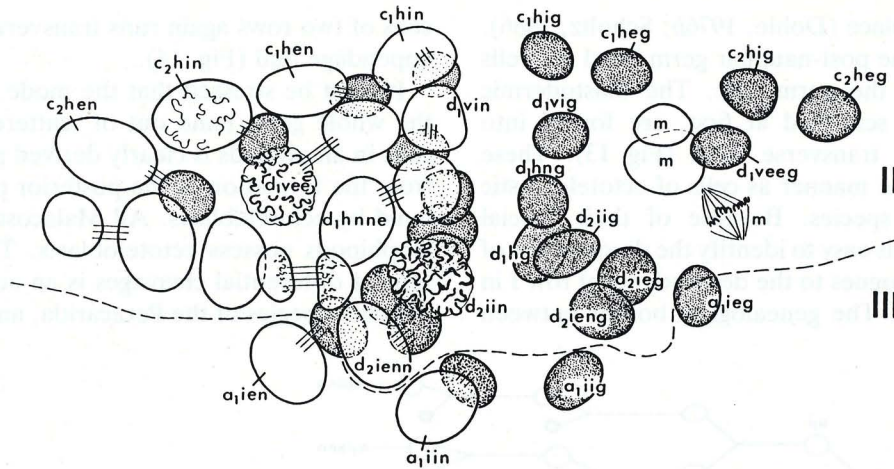


Fig. 9. The first neuroblasts and ganglion mother cells. On the animal's right side, the nuclei of the first neuroblasts are shown. They are connected with their respective ganglion mother cells by straight lines. Three lines represent the third differential cleavage, four lines represent the fourth differential cleavage. On the animal's left side, only the nuclei of the first ganglion mother cells are shown. They surround the descendants of the inner mesoderm cell mII₁. The broken line marks the genealogical boundary between derivatives of row II and row III.

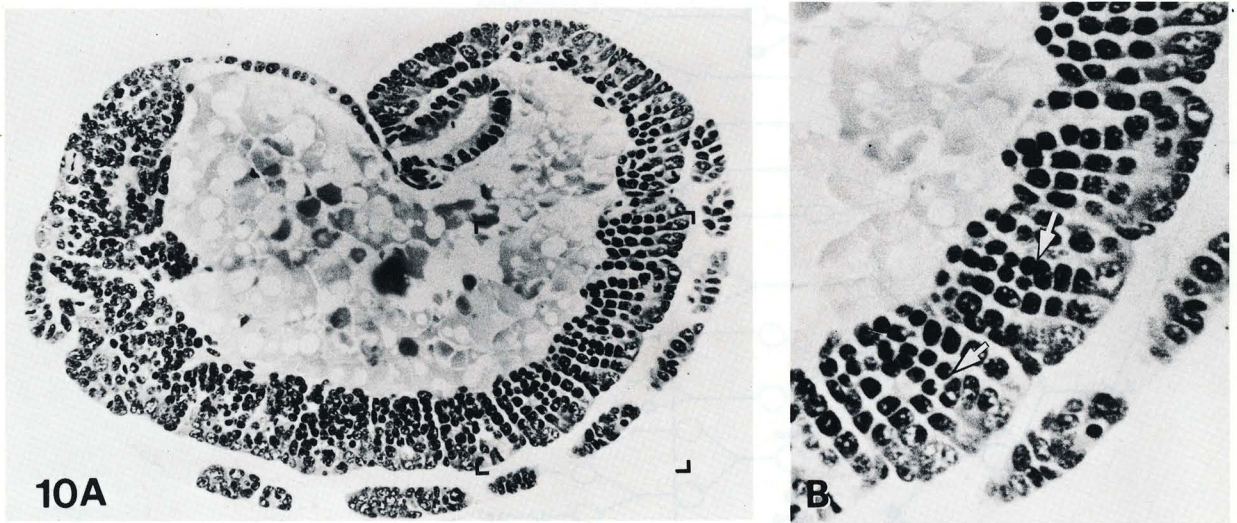


Fig. 10. Sagittal section through an advanced embryo of *Diastylis*. (A) General view. (B) Detail of A, showing ganglia with neuroblasts and rows of ganglion mother cells and ganglion cells. At the points of the arrows, the ganglion mother cells divide into ganglion cells.

the complete reduction of ectoteloblasts in amphipods.

Discussion

If the course of development of a species is characterized by stereotyped divisions and by invariant cell lineages, one is tempted to infer that one step is the inevitable prerequisite for the next step. However, the developmental process may only be a well-organized sequence of virtually independent steps. This is difficult to prove by experiments. If after

ablation of a cell the subsequent differentiation does not occur, this may be due to the fact that equivalent material cannot be substituted. Sometimes the material can be replaced in later stages. Penners (1934, 1937) showed that after destruction of ectoteloblasts in the annelid *Tubifex* the ectodermal germinal bands are missing in the embryo; in later stages the whole ectoderm can be regenerated.

Comparing the cell lineages and the differentiation processes of two or more different species, we find alternately identical and non-identical sequences. Each difference can be explained by at least one separate gene or 'instruction' which is independent of

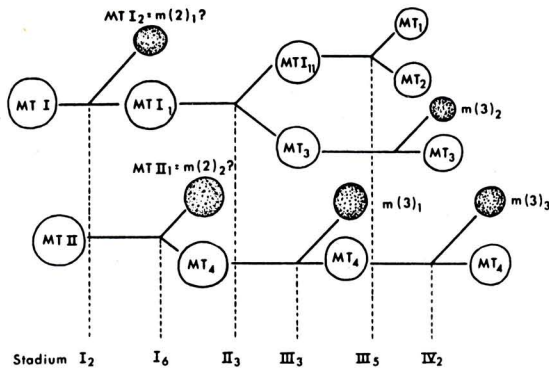


Fig. 11. Schematic representation of the divisions of the two mesoteloblast mother cells (MT I and MT II) into the four mesoteloblasts (MT₁ to MT₄) of one side. The mesoteloblast mother cells each give rise to two cells which eventually become the mesoderm cells of the second maxilla (m(2)₁ and m(2)₂). (This has not been established beyond all doubt). The cells m(3)₁, m(3)₂, and m(3)₃ will become the mesoderm cells of the first thoracic segment.

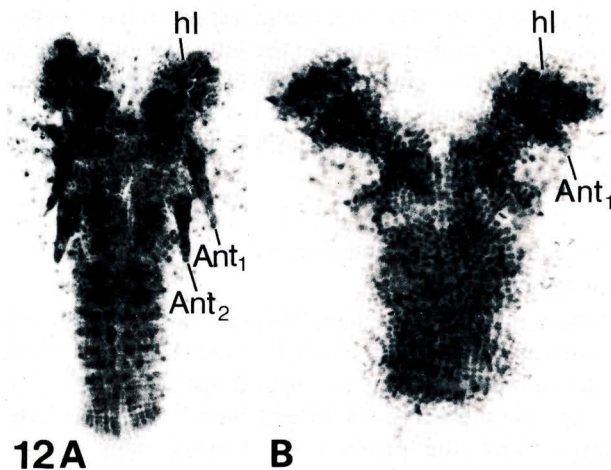


Fig. 12. Germ bands of *Neomysis* and *Diastylis*. Both germ bands represent a stage where the second differential cleavage begins in row (2) (compare Fig. 16). (A) *Neomysis integer*. The development of the two pairs of antennae (Ant₁ and Ant₂) has advanced. The eleventh row of ectoteloblast descendants has been generated. Head lobes (hl) are in an advanced stage. (B) *Diastylis rathkei*. The development of the antennae lags behind. Only vestiges of the first antennae (Ant₁) are developed. The ninth row of ectoteloblast descendants has been generated.

the programme for the preceding differentiation. In the following, an attempt is made to substantiate the independence of seemingly closely correlated morphogenetic events which are allied with segment formation in the Peracarida.

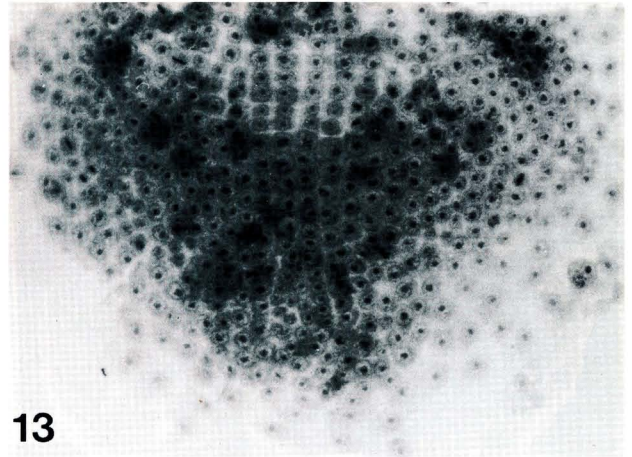


Fig. 13. Post-naupliar part of an early germ band of *Gammarus*. The cells of the germ disc have begun to arrange in rows. No ectoteloblasts are formed.

The formation of ectoteloblasts is not dependent on the genealogy of their precursor cells

In Peracarida, there is no single ectoteloblast precursor cell like the blastomere 2d in oligochaetes and leeches. In Decapoda, Oishi (1959, 1960) detected a pattern of cleavages resulting in a ring of 19 ectoteloblasts. No comparable pattern could be found in Peracarida. In tanaidaceans, the ectoteloblasts are differentiated *in situ* in front of the blastopore, whereas, in cumaceans, their differentiation begins behind the blastopore; they eventually migrate around the blastopore on both sides to meet in front of it (Fig. 1). There must be factors responsible for the differentiation of blastoderm cells into ectoteloblasts irrespective of their descent.

The arrangement of cells in iterated rows is independent of their proliferation from ectoteloblasts

Former students of germ-band formation in the Peracarida (e.g. Bergh, 1893; McMurrich, 1895; Manton, 1928; Scholl, 1963) believed that the grid-like pattern and the arrangement of cells in rows were due to the activity of the ectoteloblasts. However, rows are also formed in front of the ectoteloblast descendants in most Peracarida (Fig. 2). In Amphipoda, the cells of the germ disc are arranged in rows without the action of ectoteloblasts (Fig. 13). On the other hand, in tanaidaceans the first two rows of ectoteloblast descendants are budded off without exact order. There must be a matrix forcing the cells into a grid-like pattern regardless of the origin of the cells.

The mediolateral gradient is not a consequence of the mitotic wave in the ectoteloblasts

A wave of mitoses running from the median part to the sides of the germ band can be established not only

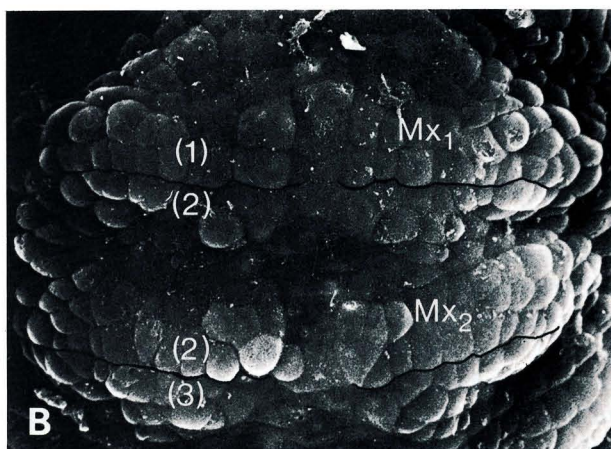
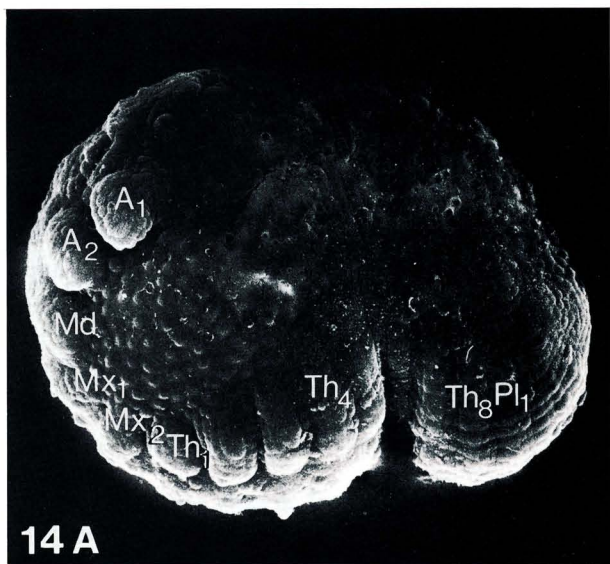


Fig. 14. SEM photographs of germ bands of *Gammarus*. (A) General view. First and second antennae (A_1 and A_2), mandibles (Md), first and second maxillae (Mx_1 and Mx_2), and the thoracic segments (Th_1 to Th_8) as well as the pleon segments (Pl_1 etc.) are formed. There is a ventral furrow between the fifth and the sixth thoracic segments. (B) Detail showing the developing first and second maxillae (Mx_1 and Mx_2). The genealogical boundaries between cells of rows (1) and (2) as well as between (2) and (3) are drawn. The boundaries run transversely over the appendage buds.

in the ectoteloblasts and their descendants, but also in the rows of non-ectoteloblastic origin. The summit of the gradient lies on both sides of the midline, at a distance of approximately one and a half cells. The gradient may be more or less steep. In insects, there is a comparable mediolateral gradient of differentiation in the germ band as first revealed by Bock (1939) and since confirmed by many authors. The gradient cannot be correlated either with the formation of cell rows or with ectoteloblasts. The mediolateral gradient, as well as an anteroposterior one, is an old

arthropodian heritage. The gradient is not a consequence of the formation of cell rows and ectoteloblasts, but, on the contrary, it persisted when teloblasts evolved in Malacostraca.

The anteroposterior gradient is not due to the age of the proliferated cells

It may seem at first sight that an anteroposterior gradient of differentiation is the result of the fact that anterior rows of cells are budded off by the ectoteloblasts earlier than the posterior ones. However, in *Diastylis* the cells of row (2) cleave first although this row is arranged later than rows (3) and I. In *Gammarus*, where no ectoteloblasts are present, a well-defined gradient also exists. Behind row (2), an anterior row always cleaves earlier than a posterior one.

An anteroposterior gradient can also be found in insects. In species with a long germ band, the nuclei of the cells that will later make up the ventral side migrate into the periplasm at the same time. Nevertheless, there is a well-defined differentiation centre usually in the region of the second maxilla.

It must be stressed that the segments in front of the first maxilla cannot be under the influence of the same anteroposterior gradient. The differentiation of the first and second antennae in the Peracarida is not correlated with the differentiation of the posterior germ band (Fig. 12).

The pattern of cleavages is not a consequence of the lineage of cells

If the cells of the post-naupliar germ band of *Diastylis* including the cells of rows (2) and (3) were all of ectoteloblastic origin, we would speculate that the complicated pattern of differential cleavages is connected with the production of small cells by the ectoteloblasts. In leeches, Zackson (1984) compared a teloblast to a stamping press and assumed that 'the iterative process of producing primary blast cells leads to the formation of the iterated segmentation pattern'. This assumption cannot be true for the Peracarida. Factors responsible for a particular cleavage pattern are not restricted to the teloblast lines. They are effective in cells of non-ectoteloblastic origin as well. By analysing the slight differences in the cleavage characteristics, we come to the conclusion that nearly every cleavage can be altered irrespective of the origin of the cell and of the surrounding pattern. The cleavage pattern is a mosaic of highly coordinated but basically independent decisions. This will not be considered in detail here. However, we must become accustomed to the idea that complex cleavage patterns may not be fundamental but merely a complicated way of distributing

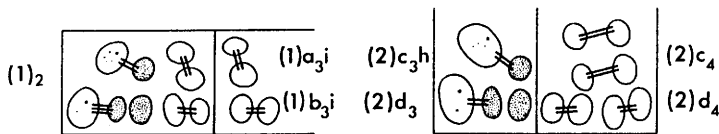


Fig. 15. The cell pattern at the apices of the first maxilla (left) and the second maxilla (right) in *Diastylis*. The pattern is exactly the same, but the cleavages which have produced the pattern are different.

and generating competent material for subsequent differentiation.

A homologous pattern of cells can be generated by cells of different origin and in different ways

This notion, which is based on the preceding discussion, may not conform to our expectation. However, it is an inevitable consequence of the fact that cells of the rows (2), (3), I and subsequent rows cleave in a nearly identical manner though their origin is partly from blastoderm cells and partly from derivatives of the ectoteloblasts (Fig. 4). In *Gammarus*, the ectodermal rows cleave in the same way though none of them is generated by ectoteloblasts.

Identical patterns of cells can also be generated through completely different cleavages. The apices of the appendage buds of the first and second maxillae are marked by a triangle of three small cells which are bordered medially by two large cells and anterolaterally by eight cells arranged in two squares (Fig. 15). These patterns are formed in different ways.

Another example can be found in the mesoderm of the first thoracic segment. The second and subsequent thoracic segments are provided with four pairs of primary mesoderm cells which originate from the four pairs of mesoteloblasts. The first thoracic segment is provided with only three pairs of primary mesoderm cells. The median pair is missing. This pair is contributed later on by mesoderm underlying the second maxilla. Two cells migrate in the posterior direction and occupy exactly the place where, in more posterior segments, the progeny of the median mesoteloblasts can be found (Fig. 16). The first unequal divisions of these contributed cells are comparable to those of cells originated from the median mesoteloblast pair.

The intersegmental furrows do not correspond to genealogical limits

An intersegmental furrow does not mark the limit between cells of two clones, but it runs transversely and slightly obliquely through cells derived from the cells of one row. This is true for all investigated Peracarida. Thus, the intersegmental furrow can have the property of a compartment boundary only after its formation.

Recently, evidence has been gathered for the existence of primary units on the germ band of *Drosophila*, which have been called parasegments (Martinez-Arias & Lawrence, 1985). The parasegments include the P (posterior) compartment of an anterior segment and the A (anterior) compartment of the subsequent segment. The parasegments seem to correspond to the units formed by the progeny of one transverse row of cells in the Peracarida. However, it must be stressed that the limits that are respected by the cell polyclones in *Drosophila* are the compartment boundaries. The parasegment cannot be defined on the basis of common descent from founder cells.

The differentiation of neuroblasts cannot be the consequence of a specific cleavage pattern

It is a special feature in Peracarida that the formation of neuroblasts and ganglion mother cells can be determined through their pedigree. We think that cells with the specifications neuroblasts and ganglion mother cells could be generated in a wholly different manner. Though we cannot demonstrate this in the Peracarida, it becomes evident by a comparison with the insects. In grasshoppers, a fixed number of neuroblasts per segment is differentiated (Doe & Goodman, 1985a). If neuroblast precursors are ablated, other neural ectodermal cells can replace them (Doe & Goodman, 1985b). The neuroblasts are determined by cell interactions, not by their lineage. In other insects, as in *Carausius*, the number of neuroblasts and their increase differs from *Schistocerca* (Tamarelle *et al.* 1985). Astonishingly enough, the formation of neuroblasts must have evolved convergently in insects and in malacostracans. In the closest relatives of the insects, the myriapods, the ventral ganglia are formed by an invagination process without differentiation of neuroblasts or columns of ganglion mother cells (Tiegs, 1940, 1947; Dohle 1964, 1974). The peculiar feature that the descendants of one blast cell contribute to the formation of two subsequent ganglia can also be found in leeches (Weisblat & Shankland, 1985; Shankland, 1987a,b).

The appendage bud is composed of parts of different clones

Parts of six to eight different cell clones are involved in the formation of an appendage bud (Fig. 7). Not all the cells of a clone and mostly not even the cells of a subclone contribute to the appendage bud. If one draws a line between the descendants of adjacent rows, this line divides an appendage bud into anterior and posterior halves. It can be deduced from the experiments of Steiner (1976) that, in *Drosophila*, a

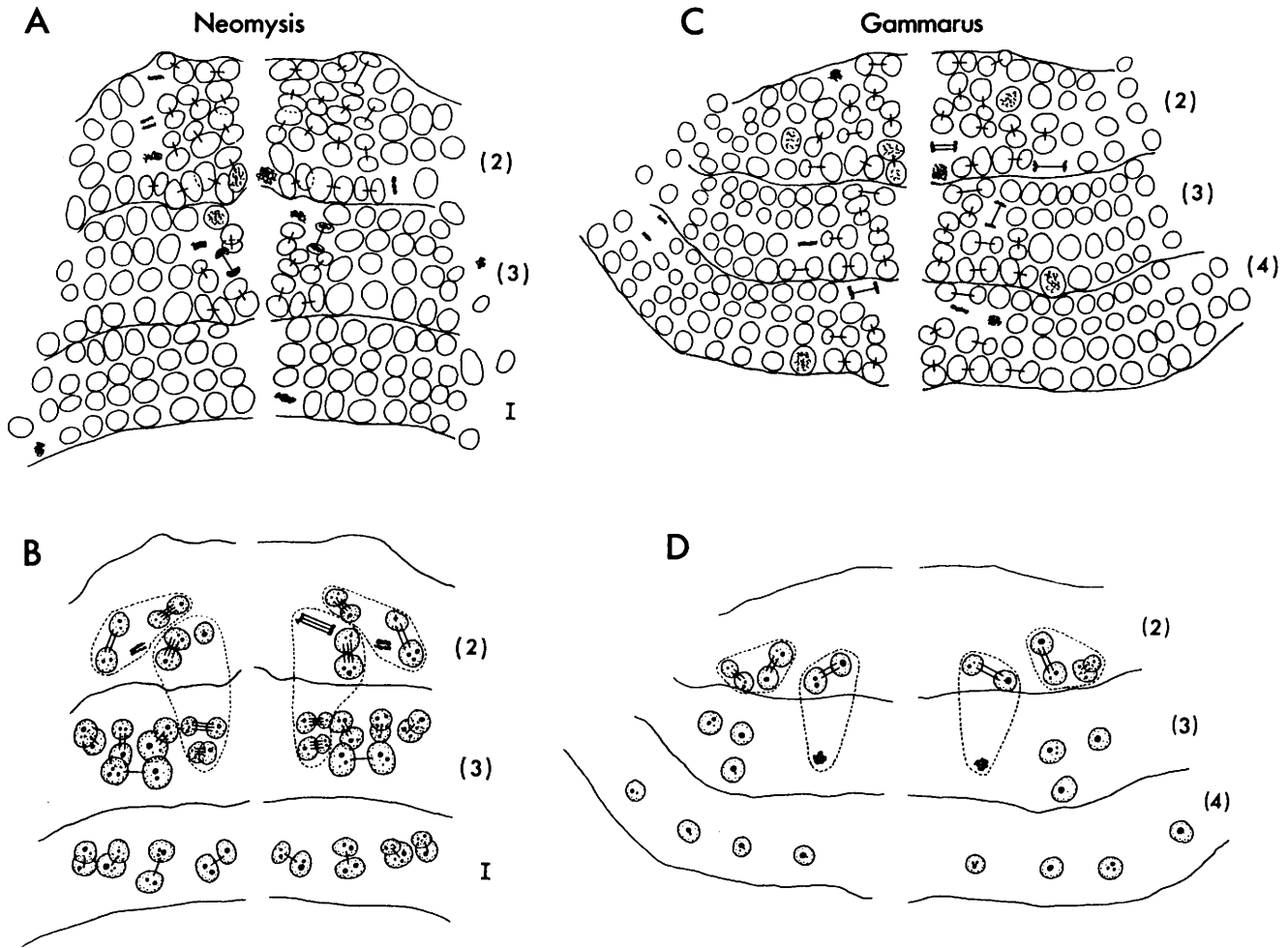


Fig. 16. Comparison of degrees of ectoderm and mesoderm differentiation in *Neomysis* and *Gammarus*. The ectoderm of row (2) is in approximately the same stage of differentiation in both cases. (A) Ectoderm of rows (2), (3) and I in *Neomysis*. The cells of the median line are omitted. Nuclei of sister cells after the first differential cleavage are connected by a straight line. (B) Mesoderm underlying the same rows in *Neomysis*. (C) Ectoderm of rows (2), (3) and (4) in *Gammarus*. Row (4) is equivalent to row I in *Neomysis*, but it is not generated by ectoteloblasts. The cells of the median line are omitted. (D) Mesoderm underlying the same rows in *Gammarus*. The mesoderm in *Gammarus* is less advanced than in *Neomysis*. The descendants of the two pairs of cells underlying row (2) and later on the segment of the second maxillae are surrounded by dotted lines. One of these cells has migrated backwards to form the inner mesoderm cell of the first thoracic segment on each side, and has already generated four cells in *Neomysis*; it is in late prophase in *Gammarus*.

genealogical limit divides the imaginal disc and later on the leg in a manner comparable to the Peracarida.

The formation of an appendage bud is not causally related to a particular cell pattern

As there is a specific cleavage pattern for the cells of each appendage bud, it could be speculated that this is responsible for the formation and differentiation of the bud. Several observations are not compatible with this assumption. In different species, the cells of the limb bud when it first clearly bulges from the surface are in quite different stages of differentiation. An identical cleavage pattern as in the preceding segments is seen in the eighth thoracic segment of

cumaceans, tanaidaceans and isopods, though this segment remains limbless in the first postembryonic stage (manca-stage). The bulge of the mandibles is formed in the same way as are the first and second maxillae, though the cells composing the mandibles are not in any discernible array.

In other arthropods, there is no clear spatial order of cells. Nevertheless, limb bud formation in insects or myriapods is homologous to limb bud formation in crustaceans. The genes for limb bud formation are phylogenetically older than those responsible for a particular cleavage pattern in Peracarida.

The conclusion of this consideration is that the invariant cleavage pattern is only a very complicated

way of generating competent material for the formation of limb buds.

The degree of differentiation in the ectoderm is not strictly correlated with that in the mesoderm

Comparison of germ bands of different peracaridan species that show the same level of differentiation in the ectoderm of a given segment reveals that ectoderm and mesoderm development is not closely correlated. In Fig. 16 parts of germ bands of *Neomysis* and *Gammarus* are shown in which the second differential cleavage in the ectodermal row (2) has started. In *Neomysis*, many more divisions have taken place in the mesoderm than in *Gammarus*. These results imply that there is no direct inductive influence of the mesoderm on the differentiation of the cell pattern in the ectoderm, and *vice versa*.

General conclusions

The formation and differentiation of segmentally repeated structures are brought forth by a cascade of processes which are normally closely linked. The impression that they are causally related seems to be justified in many cases. A comparative analysis reveals that most of these processes must have an independent genetic basis which can be altered without great effect on subsequent differentiation. For instance, the amphipods represent 'mutants' defective of ectoteloblast formation; the arrangement of cells on the post-naupliar germ band in transverse and longitudinal rows and their differential cleavages remain nearly identical to those observed in representatives of closely related orders that are provided with ectoteloblasts.

Many authors believe that an invariable cleavage pattern plays a causative role in subsequent differentiation. Sternberg & Horvitz (1981) wrote: 'One striking characteristic of these lineages – a strong correlation between lineage history and cell fate – has led to the suggestion that a specific pattern of cell divisions may be necessary for the generation of a particular cell type'. Jackson (1984) suggested 'that a specific cell division sequence might be required to generate a specific cell type'. A closer inspection of the results on nematodes and leeches presented by these and other authors rather points to the opposite conclusion. After a set of complicated cell divisions many cells still have the potential for generating a variety of cell types. In nematodes, muscle cells can be generated from the founder cells AB, MS, C and D. Neurones are differentiated by progeny of the cells AB, MS and C (Sulston *et al.* 1983).

In leeches, each of the ectodermal blast cells of the four bandlets still contributes to CNS, glia, peripheral neurones and epidermis (Shankland, 1987a,b).

Very often a specific cell type is phylogenetically much older than the division pattern by which it is generated. In the Peracarida, the earliest events in ontogeny are phylogenetically the youngest. The old heritages are – in rough phylogenetic sequence – differentiation of neurones without neuroblasts, formation of an ectodermal proliferation zone without particular blast cells, formation of intersegmental furrows without correlation to a cell pattern, formation of segmental ganglia by invagination and formation of limb buds by outpouching of an ectodermal layer with cells distributed at random.

The generation of defined ectoteloblasts, of cell rows and of a complex cleavage pattern on the post-naupliar germ band with a defined cleavage of neuroblasts, are later acquisitions which led to similar results to those of the old modes of formation. One cannot say that specific divisions cause specific differentiations. One must rather say that *in spite of* the alteration of cleavage patterns, homologous differentiations are generated. We are sure that it will be revealed, by careful comparative analyses, that this notion is true in many other cases with 'determinative' development, such as in leeches, nematodes or ascidians.

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