Spatial organization of tubercles and terrace lines in *Paradoxides forchhammeri* – evidence of lateral inhibition

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Spatial statistics on the positions of trilobite tubercles indicate the existence of a developmental spacing mechanism. Similar spacing between sensory bristles, due to lateral inhibition, is well known in insects, and the genetic basis for these patterns has been thoroughly studied. Tubercles (granules) in the Middle Cambrian trilobite *Paradoxides forchhammeri* are spaced out, but otherwise randomly positioned. Assuming that similar genetic principles are in operation for the positioning of peripheral neuronal elements in all arthropods, it can even be speculated that genes with functions similar to *Delta*, *Notch*, *achaete* and *scute* were active in trilobite cuticular patterning. Also, in *P. forchhammeri*, terrace lines (ridges) seem to display transitions into granulation, indicating that these two types of structure share an underlying pattern formation mechanism.

Key words: Trilobites, tubercles, terrace lines, pattern formation, evolution, Notch.

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Introduction

Trilobite tubercles (Størmer 1980; Wilmot 1991; Whittington 1997) are cuticular structures that may have formed in connection with sensory devices such as setae or other mechano- or chemoreceptors. As such, they may be compared with sensilla in the cuticles of other arthropods (Størmer 1980). In particular, the molecular basis for the development of spaced bristles in *Drosophila* has been the subject of extensive research (reviewed in Simpson *et al.* 1999). In insects, bristles are generally found to be placed in spacing patterns, that is, they are more dispersed than what would be expected from a totally random Poisson process (Wigglesworth 1940). The mechanism responsible for this is lateral inhibition, where bristle precursors emit an inhibitory sig-

nal to stop other bristles from forming nearby (Lawrence 1992; Simpson 1990). This signalling is based upon pathways involving the Delta and Notch gene products (Lawrence 1992; Artavanis-Tsakonas *et al*. 1995; Collier *et al*. 1996). Considering the degree of conservatism in molecular developmental pathways, it is not unreasonable to speculate that the spacing of other arthropod sensory organs, including trilobite tubercles, was under control of similar systems.

Simpson *et al*. (1999) describe possible evolutionary trends in the spacing of bristles in Diptera. They suggest that the primitive state is a spaced, but otherwise random pattern, controlled by Notch-mediated lateral inhibition. In more evolved groups, bristle patterns are more organized. Bristles only form in special domains called proneural clusters (often organized in stripes), characterized by expression of the *achaete* and *scute* genes. Within these domains, lateral inhibition is still responsible for positioning the individual bristles. The upstream regulation of *ac-sc* is not well known, but the evolution of more organized patterns would seem to require the addition of new regulatory pathways, constricting expression of *ac-sc* to specific areas. If a similar trend towards more organized tubercle patterns could be found in trilobites, it would provide an interesting example of parallel evolution between trilobites and dipterans, possibly even involving similar genes.

Statistical tests for spacing patterns

A number of statistical methods have been developed to test whether points are placed totally unorganized or if they are clustered, spaced or otherwise display ordered aspects in their positions. Such tests are described by Brown & Rothery (1993) and Davis (1986). A comparison between several different statistical methods for classifying spacing patterns is given by Wallet & Dussert (1997). The problem is more involved than might be expected, and most tests seem to fail in some more or less pathological cases.

Most tests can be classified into either quadrat analysis or nearest neighbour methods. The nearest-neighbour methods are more robust (Davis 1986), and will be used here. The description below follows Davis (1986) with minor modifications.

For a random pattern without spacing or clustering (Poisson process), the distances from each point to its nearest neighbour follow an exponential distribution with mean

$$\bar{\delta} = \frac{1}{2}(A/n)^{1/2}$$

where A is the area to be studied and n is the number of points. The sampling variance $\sigma_{\bar{\delta}}^2$ of $\bar{\delta}$ is given by

$$\sigma_{\bar{\delta}}^2 = \frac{(4-\pi)A}{4\pi n^2}$$

The standard error of the estimate of the mean distance between nearest neighbours is

$$s_e = (\sigma_{\bar{\delta}}^2)^{1/2} = \frac{((4-\pi)A)^{1/2}}{2\pi^{1/2}n}$$

Given these theoretical values for the mean and standard error of the nearest neighbour distances for an unorganized random pattern, the simple Z test can be used to test whether the observed mean distance $\bar{d}$ is equal to the value expected for an unorganized pattern. We are then in a position to set up and possibly reject the null hypothesis that the observed pattern is unorganized (Poisson process).

Also, one can compute the so-called nearest-neighbour statistic

$$R = \bar{d} / \bar{\delta}$$

which ranges from 0.0 for a distribution where all points coincide, to 1.0 for a random distribution of points, up to a maximum of 2.15 for a maximally spaced pattern where the points are placed in a hexagonal grid. Values of R for different spacing patterns are given in the literature. Claxton (1963) measured $R = 1.67$ for skin follicles in sheep, Lawrence & Hayward (1971) got $R = 1.4$ for bristles in the hemipteran *Oncopeltus*, Claxton (1974) reported $R = 1.37$ for bristles in *Drosophila*, Holder & Glade (1984) got $R = 1.64$ for skin glands in the axolotl and Larkin *et al.* (1996) found $R = 1.40$ for trichomes ('hairs') on the leaves of *Arabidopsis*. All these authors propose a lateral inhibition mechanism for the spacing (see especially Holder & Glade (1984) for a thorough discussion).

Using the device of Minimal Spanning Trees (Dussert *et al.* 1988), it is possible to provide other statistics for the spacing of trilobite tubercles. The MST method can be more sensitive than nearest neighbour statistics in some respects (Wallet & Dussert 1997), and it provides a check on the results above.

The MST is a set of lines connecting all the points, such that there are no loops in the set, and such that the sum of line lengths is the smallest possible. The mean m and standard deviation σ of line lengths provide parameters to determine the amount of overdispersion of the pattern.

Also, the pattern can be compared with an unorganized reference data set using appropriate scaling techniques (Hoffman & Jain 1983).

Dussert *et al.* (1988) suggest using the MST method for determining directional properties of point distributions. In particular, plotting the lengths of the lines of the MST against their angles with respect to the horizontal can bring out anisotropic features that are difficult to see otherwise. Such a plot is called a Developed Angular Diagram (DAD). The DAD can only be used for relatively large data sets, preferably more than 100 points. If tubercles and terrace lines share some pattern formation mechanisms (see below), it would be possible that tubercles show a tendency for alignment in stripes. The DAD is not able to show such alignment if the stripes are oriented in all directions, but can bring out linear features if they are preferentially oriented in a single direction.

Organisation of tubercles in *Paradoxides forchhammeri*

Two flat regions of a granulate thorax pleura (Fig. 1) from *Paradoxides forchhammeri* Angelin, 1854, Middle Cambrian, Krekling, Norway (Brögger 1878; Bergström & Levi-Setti 1978), were drawn using a camera lucida. The drawings were digitized, and the coordinates of the tubercles determined. It was also tried to measure the positions of tubercles from photographs, but this invariably gave large errors when compared to

Fig. 1. Left thorax pleura from the trilobite *Paradoxides forchhammeri*, Middle Cambrian, Krekling, Norway (PMO164.581); × 1.75. See also Fig. 2.

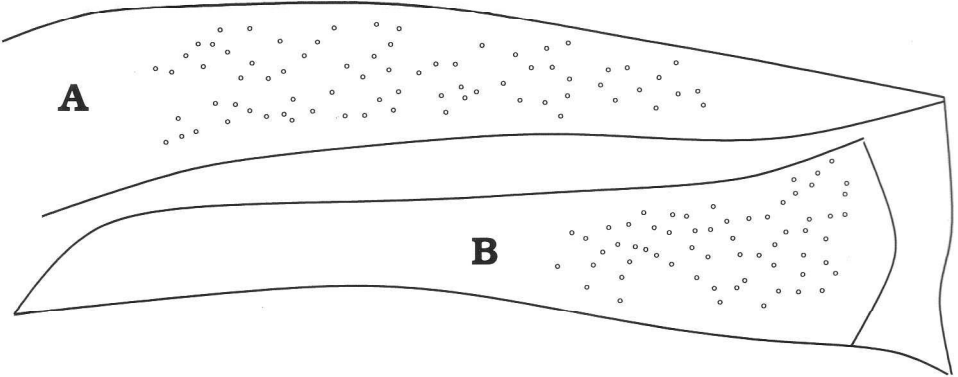Fig. 2. Digitized proximal region of a left thorax pleura from the trilobite *Paradoxides forchhammeri* (Fig. 1); × 2.5. **A**. Band anterior to pleural furrow. $N = 74$, $Z = 5.99$, $R = 1.32$, $\bar{d} = 0.86$ mm. **B**. Posterior band of the same pleura. $N = 61$, $Z = 6.34$, $R = 1.38$.

the original specimen. In photographs, some tubercles become invisible, spurious bright spots can be erroneously interpreted as tubercles, and positions can be inaccurate. In *P. forchhammeri* the tubercles are practically points, with very large distances between tubercles compared with their diameters. Tubercles near the edges were discarded from the nearest-neighbour search to eliminate boundary effects (Davis 1986). The results are shown in Fig. 2, and an unorganized distribution can be rejected at $p < 0.005$. The nearest-neighbour statistics are $R = 1.32$ and $R = 1.38$, showing that the tubercles are overdispersed. In a Monte Carlo test, 10000 random test patterns were generated, with the same numbers of points as in Fig. 2 distributed uniformly and independently (Poisson process) in similar geometries. Not a single pattern showed a smallest nearest neighbour distance as large as the smallest nearest neighbour distance in the data set, again strongly indicating a spacing pattern.

A number of fragments of *P. forchhammeri* from the Brögger (1878) collection in the Paleontological Museum, Oslo, were also measured. These specimens are from the same locality as the one in Fig. 1, but the precise horizon is not known. The patterns in these are still significantly overdispersed (Figs. 3–5). The values of R are between 1.3 and 1.4.

It should be noted that most possible sources of error would decrease the value of R. Non-systematic errors in measurement would randomize the patterns, decreasing the R value. Bad preservation might cause granules to be missing, producing gaps. This is equivalent to a clustering of the remaining points, decreasing the value of R. Also, we are observing patterns on a scale which includes empty areas (possible muscle attach-
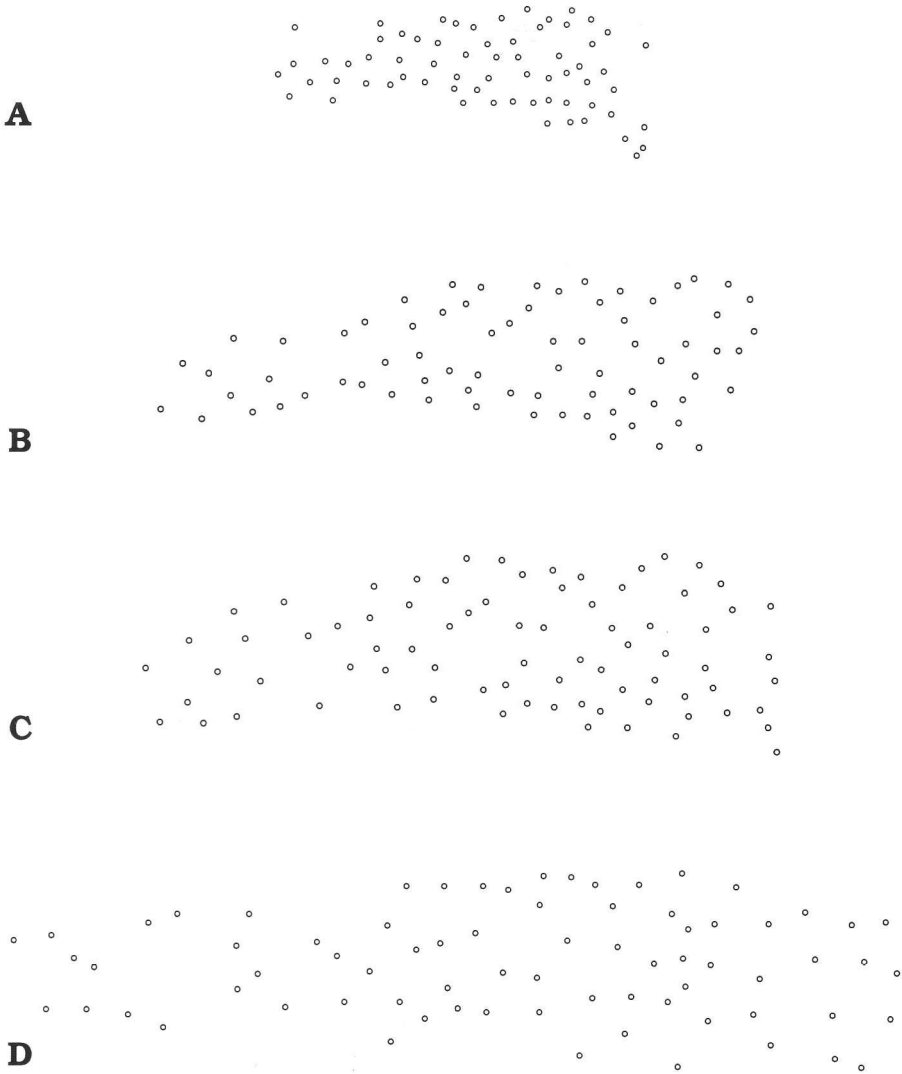
Fig. 3. Digitized regions of *Paradoxides forchhammeri* thorax fragments. The null hypothesis of a Poisson process can be rejected at $p < 0.005$ in all cases; $\times 4.6$. **A**. Right pleura, anterior band (PMO28573). $N = 66$, $Z = 6.3$, $R = 1.36$, $\bar{d} = 0.47$ mm. **B**. Partial right pleura, anterior band (PMO28578). $N = 72$, $Z = 7.0$, $R = 1.38$, $\bar{d} = 0.62$ mm. **C**. Partial right pleura, anterior band (PMO28595). $N = 78$, $Z = 6.8$, $R = 1.36$, $\bar{d} = 0.67$ mm. **D**. Left pleura, posterior band (PMO28602). $N = 74$, $Z = 7.0$, $R = 1.38$, $\bar{d} = 0.87$ mm.

ment sites). These areas would again reduce the value of R. The values given here are therefore conservative with respect to the hypothesis of overdispersion. Another source of error might be the estimation of total area, which is being measured somewhat crudely by the area of a polygon drawn just outside the 'outermost' tubercles. The selection of tubercles to include as vertices in this polygon is sometimes a little arbitrary, but it is not expected that the measured values of R be seriously affected by this. In particular, the quadratic geometry of the left fixigena in Fig. 5 should be relatively
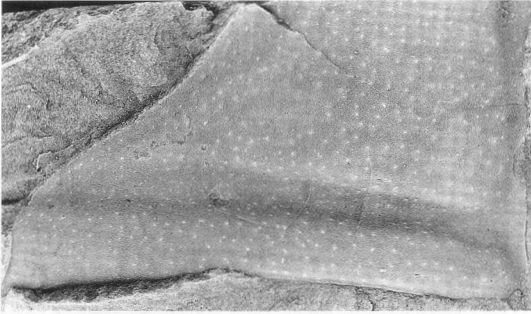
Fig. 4. Partial cranidium from the trilobite *Paradoxides forchhammeri*, Middle Cambrian, Krekling, Norway (PMO28606); × 2.5. See also Fig. 5.
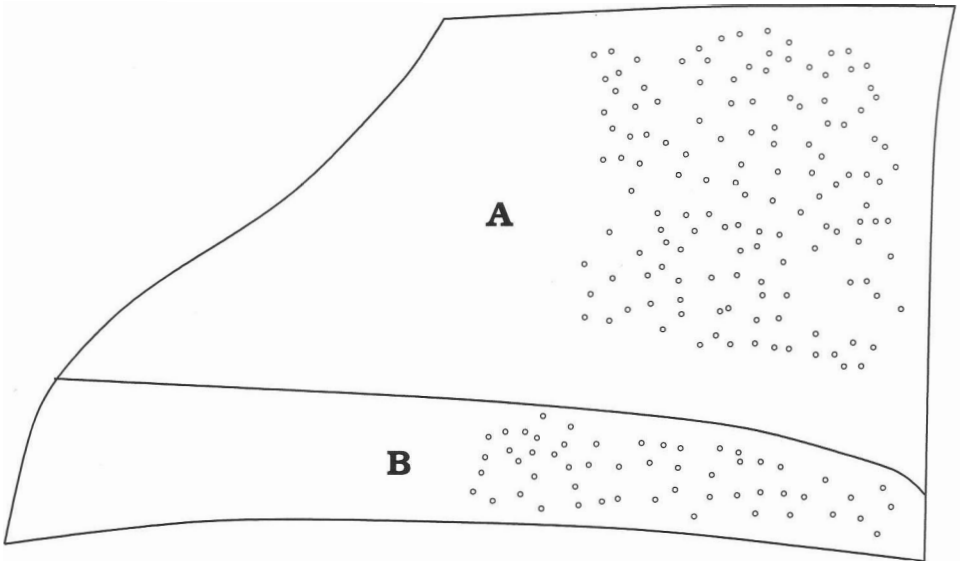

Fig. 5. Digitized regions of the cranidium of *Paradoxides forchhammeri* (Fig. 4). The null hypothesis of a Poisson process can be rejected at $p = 0.005$ in both cases; × 4. **A.** Partial left fixigena, postocular area (PMO 28606). $N = 136$, $Z = 7.8$, $R = 1.31$, $\bar{d} = 0.76$ mm. **B.** Left posterior border (PMO28606). $N = 54$, $Z = 6.7$, $R = 1.42$, $\bar{d} = 0.79$ mm.

insensitive to this problem, and the R value is in reasonable accordance with the other measurements in this case. A less subjective area estimation using the convex hull of the points (Hoffman & Jain 1983) is not applicable in this case, because it seriously overestimates the area of concave domains.

The Minimal Spanning Tree technique of Hoffmann & Jain (1983) was also carried out in order to test for overdispersion. With this procedure, a random Poisson process could be rejected at $p < 0.001$ (Kolmogorov-Smirnov).

These spacing patterns can be explained by a lateral inhibition mechanism, with a signal from existing tubercles preventing the formation of other tubercles nearby. Since the number of tubercles increased during growth (Tripp *et al.* 1977), it can be assumed that the new tubercles formed in positions where the distance to old tubercles
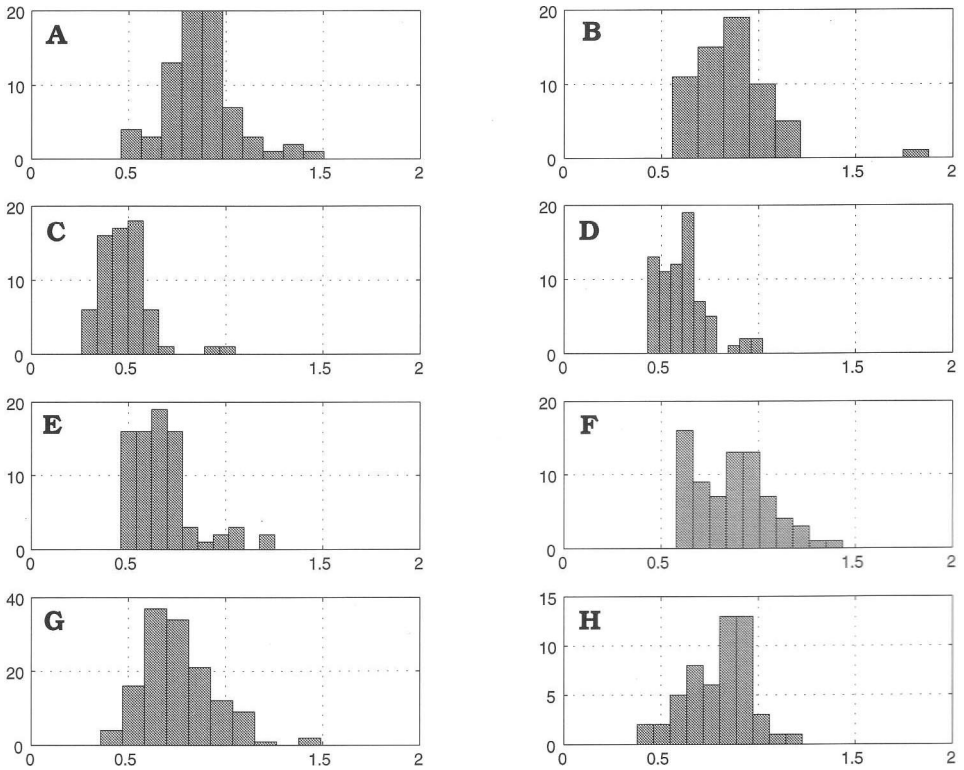
Fig. 6. The distribution of nearest neighbour distances between *Paradoxides* tubercles. **A**. Left thorax pleura (PMO164.581), band posterior to pleural furrow. $N = 74$, $\bar{d} = 0.87\pm0.18$ mm. **B**. Anterior band of same. $N = 61$, $\bar{d} = 0.86\pm0.21$ mm. **C**. Right pleura, anterior band (PMO28573). $N = 66$, $\bar{d} = 0.47\pm0.13$ mm. **D**. Partial right pleura, anterior band (PMO28578). $N = 72$, $\bar{d} = 0.62\pm0.13$ mm. **E**. Partial right pleura, anterior band (PMO28595). $N = 78$, $\bar{d} = 0.67\pm0.16$ mm. **F**. Left pleura, posterior band (PMO28602). $N = 74$, $\bar{d} = 0.87\pm0.20$ mm. **G**. Partial left fixigena (PMO28606). $N = 136$, $\bar{d} = 0.76\pm0.20$ mm. **H**. Left posterior border (PMO28606). $N = 54$, $\bar{d} = 0.79\pm0.17$ mm.

had become sufficiently large due to the expanding geometry. A similar idea was hypothesized by Tripp *et al.* (1977) for the tubercles on the pygidium of *Encrinurus variolaris*, that there are interactions between the rings which inhibit the development of tubercles on adjacent rings and favour their development on alternate rings.

The distributions of nearest neighbour distances for the *Paradoxides* specimens above are given in Fig. 6. By studying these distributions, further information about the nature of the lateral inhibition process may be gained.

One way in which the inhibition process could work would be by sequential lateral inhibition (Brown & Rothery 1993). This means that the points are added in random positions in sequence, but with an inhibiting signal being emitted from existing points such that attempts to add points within a certain inhibitory radius are rejected. This simple and intuitive model does not fit the *Paradoxides* data. To see this, we note that it would not matter much for the nearest neighbour distribution whether close points are rejected immediately, or points are added regardless of distance (in a Poisson process) and close points removed in a second pass. This implies that the sequential lateral inhi-
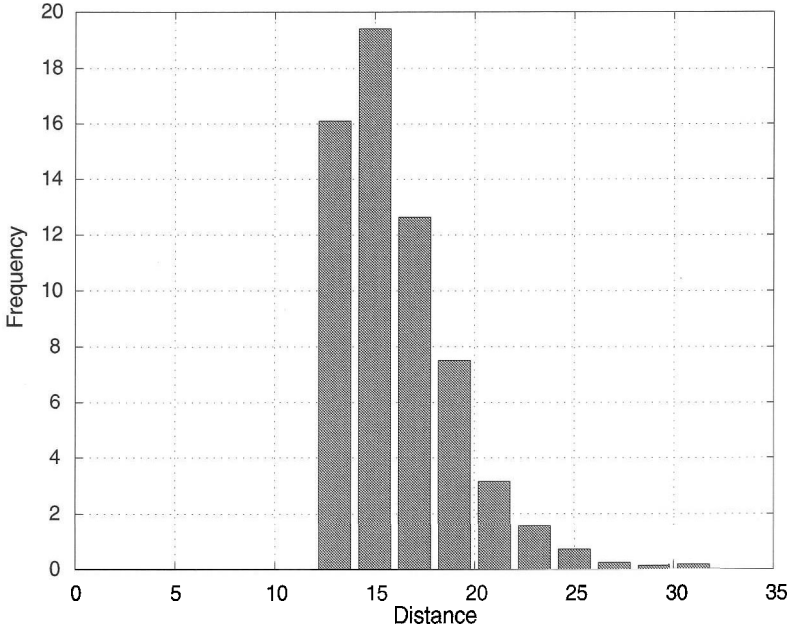
Fig. 7. The mean distribution of nearest neighbour distances in 100 simulation runs of a sequential lateral inhibition process, with $N = 61, D_{min} = 13$. Note the similarity to an exponential distribution truncated to the left.

bition model would give a similar nearest neighbour distribution as in a simple Poisson process, but where all the nearest neighbour distances smaller than the critical distance are removed. Therefore, we would expect the nearest neighbour distances from a sequential lateral inhibition process to follow an exponential distribution (as in a Poisson process), but with all frequencies below the critical distance set to zero. As an example, Fig. 7 shows the mean distribution after 100 simulation runs of a sequential inhibition process with $N = 61, D_{min} = 13$.

The null hypothesis of a sequential lateral inhibition process can be tested by comparison with the theoretical truncated exponential distribution, with parameters (minimal and mean distance) taken from the *Paradoxides* data set (Fig. 2). This hypothesis could be rejected at $p < 0.002$ (Kolmogorov-Smirnov). The real distribution seems to be more bell-shaped and less right-skewed than the null hypothesis distribution, especially if the outlier to the right is removed.

This is also seen in Fig. 8, where the empirical cumulative distribution functions for the nearest neighbour distances are presented. The distributions for the sequential inhibition simulations cannot be well fitted to the data, even when different inhibition radii are tried. The data has a tail of smaller distances, while the simulations show the expected truncation of small values.

It seems, therefore, that some other kind of lateral inhibition process must be responsible for the distance distribution. One possibility is a competitive, distributed process where points were added in a continuous fashion. A reaction diffusion-like mechanism would act in this manner, as would a lateral inhibition mechanism of the Delta-Notch type (Collier *et al.* 1996). Points were not added in random positions se-
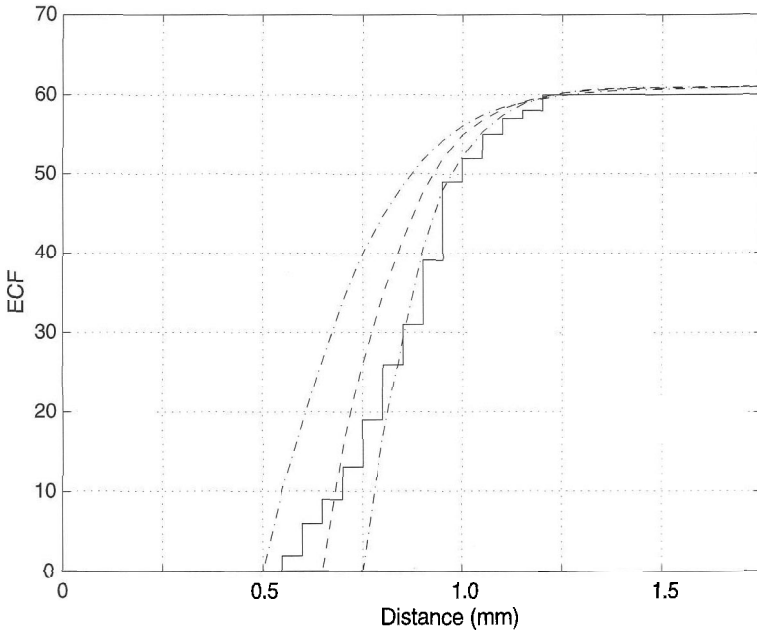
Fig. 8. The empirical cumulative distribution of nearest neighbour distances of the *Paradoxides* data set (solid line), and the mean distributions for 100 simulation runs of a sequential lateral inhibition process, with $D_{min} = 0.50, 0.67, 0.75$ mm (broken lines).

quentially. Instead, new points emerged competitively, meaning that the positions furthest from existing points would 'win', producing points which immediately suppressed new points nearby. The result was a more or less regular spacing pattern, with a symmetrical nearest neighbour distribution.

Alternatively, a sequential lateral inhibition process could be responsible for these patterns if the inhibition radius was rather variable from tubercle to tubercle.

The lateral inhibition theory implies that variation in the placement of tubercles is not to be ascribed to genetic variation, but simply to the accidents of development. Statistics on the overall distribution of points may be of taxonomical value, however. Also, the geometry of the domain is an important factor that may constrain the positions of tubercles under crowded conditions. Finally, tubercles are less common in some specific areas, presumably corresponding to muscle attachment sites, which are likely to have been under strict genetic control.

Another possibility that must be discussed is that the position of every tubercle was directly coded in the genome. As we have seen, this would be in conflict with current views in developmental biology, and to code all the tubercles in Fig. 2 individually would clearly be inefficient. However, some of the larger tubercles on *Encrinurus* do seem to arrive recurrently in the same positions, which can even be used as a relatively stable taxonomical criterion (Tripp 1957; Tripp *et al.* 1977). A number of smaller, adventitious tubercles form in variable positions between the larger tubercles. Interestingly, a similar pattern is seen in *Drosophila*, where certain
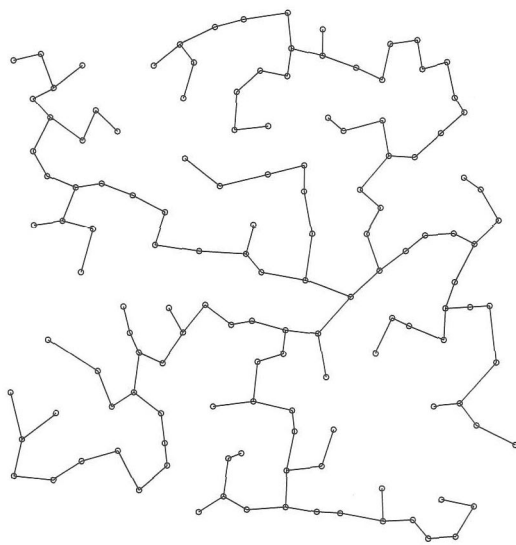
Fig. 9. The Minimal Spanning Tree for the *Paradoxides* tubercle pattern shown in Fig. 5 (partial left fixigena, PMO28606).

so-called landmark bristles always form in the same positions, presumably under some kind of hard-wired genetic control. Between these large bristles, the much more numerous smaller bristles are placed in a spacing pattern facilitated by a lateral inhibition mechanism.

Linear arrangement of tubercles

A possible arrangement of the tubercles in lines was studied using the Minimal Spanning Tree technique of Dussert *et al.* (1988). Fig. 9 shows the MST for the *Paradoxides* fixigena in Fig. 5. The Developed Angular Diagram is given in Fig. 10. The blank area to the left (at small distances) is due to the overdispersion. A tendency for stronger inhibition around the angle $\phi = 0.9$ (52 degrees counterclockwise from east) may be detected as a widening of the blank area around this angle (Dussert *et al.* 1988). This corresponds to a direction approximately parallell with the left cephalic border. Any underlying stripes, if present, would then be oriented normal to this, that is in a direction normal to the left cephalic border, in accordance with the orientation of terrace lines in the postocular area of other trilobite species (Miller 1975: fig. 10A). Unfortunately, no statistical tests for the presence of such widenings in DADs have yet been developed.

Organisation of tubercles in other trilobite genera

The statistics described in the previous section were also applied to the distribution of tubercles on a cephalon of the Ordovician trilobite *Paraszechuanella iranica*, shown in Fig. 11. These tubercles are sufficiently widely spaced compared with their diame-
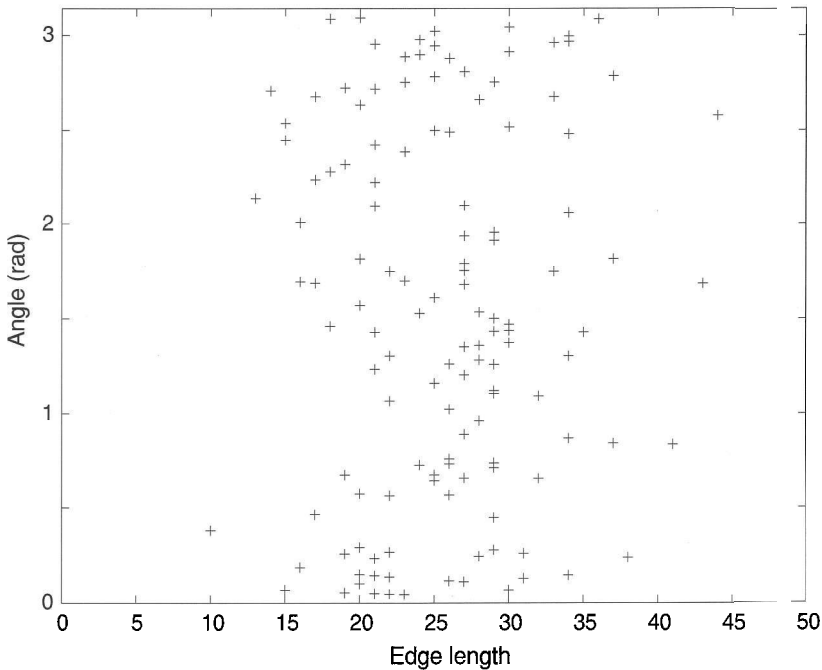
Fig. 10. The Developed Angular Diagram for the Minimal Spanning Tree in Fig. 9, plotting the angle in radians of each line of the MST against its length. Note the indentation around the angle $\phi = 0.9$.

ters that the methods should give reasonable results, although this may be questionable for the glabella, where the tubercles are most closely packed.

The co-ordinates of the tubercles in four different regions determined. To minimize the effect of distortion caused by the slightly non-planar geometry of the surface, points near the borders of the domains (where the angle between the surface and the line of sight is small) were discarded.

The positions of the tubercles are shown in Fig. 12, together with the statistics. From the given values of Z, the null hypothesis of an unorganized distribution can safely be rejected at $p < 0.005$, corresponding to a Z value of 2.8. The values of R show that the tubercles are highly overdispersed, with even higher R values than for *Paradoxides forchhammeri*.

In the Silurian *Encrinurus*, the positioning of tubercles seems to be even more strictly controlled (Tripp 1957; Tripp *et al*. 1977). It remains to be seen whether there is an evolutionary trend towards more spaced and organized tubercle patterns in trilobites, but if so, it would have a parallell in the evolution of bristle patterning in the Diptera (Simpson *et al*. 1999).

Terrace lines

Trilobite terrace lines (Miller 1975; Schmalfuss 1981) are cuticular ridges that may have increased friction during burrowing, or more probably had a sensory function.
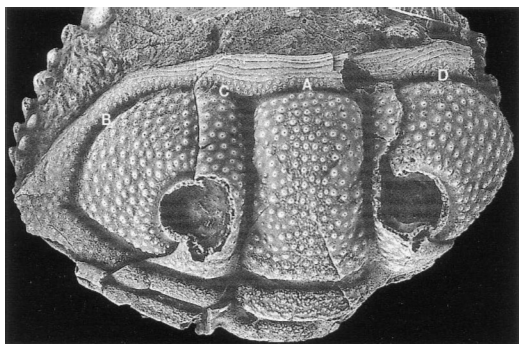
Fig. 11. A trilobite with heavy tuberculation: *Paraszechuanella iranica* (Pillet, 1973). Tremadoc, Iran; × 3. Letters refer to regions digitized in Fig. 12.
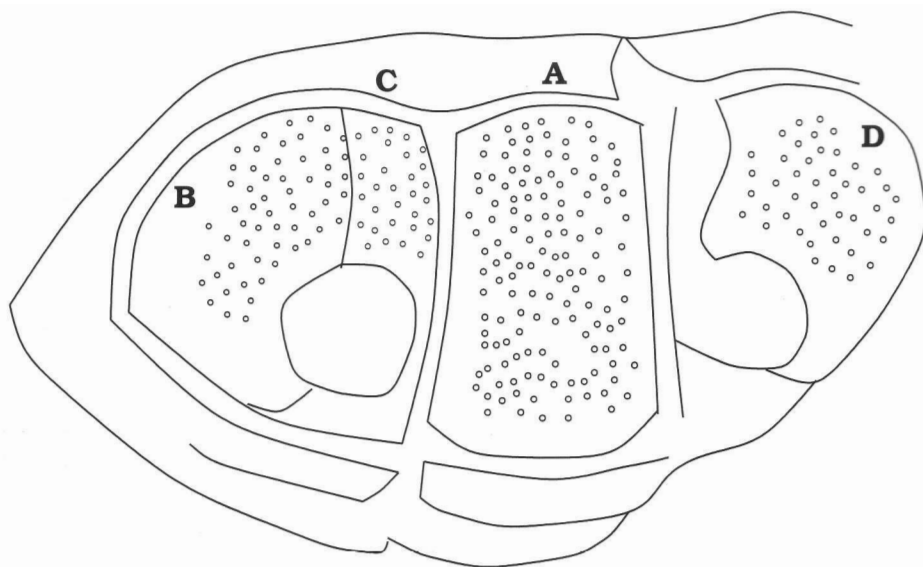

Fig. 12. Digitized regions of the trilobite cephalon in Fig. 11; × 5.3. **A.** $N = 135$, $Z = 14.37$, $R = 1.65$. **B.** $N = 57$, $Z = 11.38$, $R = 1.79$. **C.** $N = 31$, $Z = 9.81$, $R = 1.92$. **D.** $N = 45$, $Z = 9.72$, $R = 1.76$.

Fortey (1986) suggested that terrace lines (ridges) served a function in sensing the state of enrollment. Morphologically, terrace ridges and the more symmetrical raised lines consist of subparallel lines which occasionally branch or terminate. Some modern crustaceans have vaguely similar structures. The qualitative similarity with human finger prints has often been noted.

As is well known from theoretical work in biological pattern formation, overdispersed stripe and dot patterns are easy to produce in systems that include local self-activation and lateral inhibition (e.g., Meinhardt 1995; Pearson 1993; Murray 1993). Several different mechanisms might account for the lateral inhibition, including diffusion of a morphogen (reaction-diffusion models, Turing 1952; Meinhardt 1995), mechanical signalling (Murray *et al.* 1988; Odell *et al.* 1981), and cell-to-cell relay signalling (e.g., Hammer 1998).

For the illustration of pattern formation in lateral inhibition systems, some examples of reaction-diffusion models are given below. For details, see e.g., Murray (1993) and Meinhardt (1995). It should be noted that these are only meant as abstract illustrations. In the case of trilobite cuticular patterns, it might be speculated that direct cell-to-cell signalling (through Delta and Notch) is the mechanism responsible for pattern formation. Cell-to-cell signalling models can be proposed which are numerically equivalent to reaction-diffusion models, however (Hammer 1998).

One way of producing striped patterns in a reaction-diffusion system is by adding a *convection term*. This models a directional flow or streaming in the tissue, as could be induced by flow of body liquids or even flow in the external environment. Mathematically, this is introduced by adding a first-order derivative term to the reaction-diffusion equations. For flow in the positive x direction, we have

$$\frac{\partial a}{\partial t} = F(a,b) + D_a \nabla^2 a - C\frac{\partial a}{\partial x}$$

$$\frac{\partial b}{\partial t} = G(a,b) + D_b \nabla^2 b - C\frac{\partial b}{\partial x}$$

where a and b are the concentrations of two morphogens (e.g., activator and inhibitor), F and G are reaction terms, the Laplace terms model diffusion, and C is the transportation rate.

The derivatives are approximated using a difference method (for discussion of numerical methods for diffusion-convection problems, see Fletcher 1991). A typical experiment with such a reaction-diffusion-convection system is shown in Fig. 13. The stripes are oriented normal to the direction of transportation. Cyclical boundary conditions were used to produce this pattern, simulating a toroidal domain without defined boundaries. With homogenous Neumann boundary conditions (zero flux), stripes tend to become oriented in the direction of convection, rather than normal to it.

Alternatively, such stripes can be produced without convection, using special parameters in a standard reaction-diffusion model, but some assumptions have to be made in order to enforce their correct orientation. The geometry may control the orientation however. One of the most convincing examples to date of convergence between mathematical modelling of reaction-diffusion and observed biological pattern development is based on the effect of expanding geometry upon the orientation, intercalation and bifurcation of stripes, without convection (Kondo & Asai 1995). Their examples of pigmentation stripes on the angelfish *Pomacanthus* are geometrically similar to trilobite terrace lines. As mentioned, there are other mechanisms that could provide these patterns. Striped patterns with a characteristic distance and the resulting intercalation in growing domains could be explained by mechanical effects or by certain chemotactical cell migration processes (Höfer & Maini 1996). On an abstract level, all these models are similar, involving lateral inhibition.

Striped patterns can also occur in a reaction-diffusion system with anisotropic diffusion rates (Wolpert & Stein 1984), or, as suggested by Simpson *et al.* (1999), a stripe may be initiated close to a boundary because of special conditions there. Once this stripe has formed, new stripes could be initiated sequentially, in parallel orientation and with a spacing mediated by lateral inhibition.
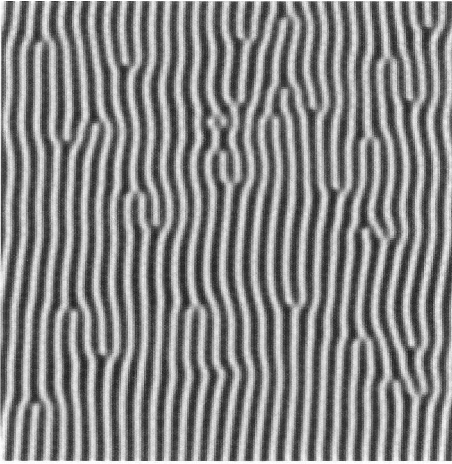
Fig. 13. Emergence of a stripe pattern using the Gray-Scott reaction-diffusion model (Pearson 1993) with convection in the x direction. $F = 0.06$, $k = 0.062$, $D_u = 0.2$, $D_v = 0.05$, $C = 0.2$ (qualitatively, the pattern is very robust to parameter changes). A 200 by 200 grid was used, and run for 3500 time steps.

Miller (1975) observed that the spacing between terrace lines is relatively constant over ontogeny, and he presents a scatter diagram of librigena size vs. terrace line spacing over a wide size range in a particular species, clearly supporting this. Schmalfuss (1981) also notes that the distance between terrace lines is relatively constant and typical for a species. When moulting, the previous lines are copied onto the new and larger exoskeleton, but new lines are introduced between the old ones to keep the constant distance. Schmalfuss (1981: p. 337) attributes this intercalation in an expanding geometry to 'a morphogenetic (or biochemical) gradient inducing the formation of new terraces as soon as a certain threshold distance between individual terraces is surpassed by growth'. This amounts to lateral inhibition. As terrace lines are assumed to be associated with neuronal function, it may even again be speculated that Delta and Notch might be involved in the process. It would be interesting to test this for 'terrace lines' in modern arthropods, though they might of course not be homologous with trilobite terrace lines.

There are other observations supporting the idea that terrace line patterns were not hard-coded in the genome, but emerged through a reaction-diffusion-like mechanism as the geometry grew over successive moulting stages. Miller (1975) illustrates a meraspis of *Paladin eichwaldi* that has not yet developed terrace lines. Sub-parallel terrace lines appear in the holaspis stage, indicating that the pattern formation system had not yet self-organized in the smaller stage.

Sub-parallel and labyrinthic, anastomosing patterns can occur in different positions on the exoskeleton of an individual. Such variation would be easy to accomplish by varying the parameters of a reaction-diffusion-like system.

And finally, the terrace line pattern is normally not bilaterally symmetrical. Differences in branching and intercalation between the left and right sides indicate a developmental system that was somewhat lax, with autonomous pattern formation on either side (Seilacher 1991), and not under direct genetic control as for example by a combinatorial code. Incidentally, terrace lines on, e.g., the pygidial doublure might provide excellent opportunities for studying fluctuating asymmetry in trilobites

(Smith 1998). Since the pattern formation process was probably autonomous on either side, such studies should consider 'bulk properties' of the pattern, such as mean distance between lines.

The possibility that the spacing between terrace lines (and also possibly tubercles) was controlled by a cell lineage mechanism is an alternative to the lateral inhibition theory. Such a clonal mechanism has been suggested for spacing between stomata in certain plants (Sachs 1991), the idea being that the stomata cells are part of a monoclonal group of cells. These cell clusters are closely packed, producing overdispersion between the stomata that are placed centrally in the cluster. There are several reasons why a cell lineage mechanism was probably not acting to space out trilobite sensory elements. As mentioned, it is known that lateral inhibition is responsible for the spacing of sensory elements in modern arthropods (Lawrence 1992). Also, insertion of new elements as the structure grows is not easily explained with a cell lineage mechanism. The distance between terrace lines is highly variable within a single specimen. For example, the lines are often packed closely together near the pygidial axis, and further spaced apart laterally. Accordingly, the spacing must have had some plasticity, being able to be regulated. A fixed number of inter-element cells, as expected in a cell lineage model (at least if one assumes constant cell size), did not exist. On the other hand, the range of lateral inhibition is easily regulated by the geometry and curvature of the domain (Spirov 1993), possibly regulation of gap junctions, cell packing, and hysteresis mechanisms that may delay activation even after a low level of inhibitor is reached. Characteristic spacing distance may be controlled by particular regulating genes. In the context of lateral inhibition, this has been shown for invagination centres in the *Drosophila* gut, being controlled by the *wingless* gene (González-Gaitán & Jäckle 1995), and trichomes in *Arabidopsis*, being controlled by the *rtn* gene (Larkin *et al.* 1996). A search for different spacing alleles for trilobite tubercles and terrace lines might therefore be of interest.

Still, some of the dispersed sensory bristles in *Drosophila* are arranged in rows, and the spacing between rows does not seem to be due to simple lateral inhibition but rather to a hierarchical process involving some of the same gap genes (i.e., *hairy*) that produce early segmentation (Orenic *et al.* 1993, but see also Simpson *et al.* 1999 for the suggestion that these mechanisms may have evolved from Notch-mediated lateral inhibition between stripes). But again, such a mechanism would not explain the insertion of new elements during growth in trilobites.

Another interesting aspect of trilobite terrace lines is their polarity. Terrace lines are transversally asymmetrical, with a 'slope' and a 'scarp' side. The sensory bristles in *Drosophila* show similar polarity, pointing in a well-defined direction. The main theory for the polarity of *Drosophila* bristles is that they are developing in a field with a morphogenetic gradient, and that the developmental system is able to sense the direction of this gradient (Lawrence 1992; Struhl *et al.* 1997a, b). This may have been the case for trilobite terrace lines as well, raising interesting problems regarding the basis for different gradients directed radially in the hypostome, laterally on the pygidium etc. Miller (1975: fig. 13C) illustrates thorax segments of *Illaenus sarsi* where the polarity of terrace lines reverses laterally, after a break in the slope of the segment. This indicates a longitudinally oriented boundary (e.g., Lawrence 1992) at the break, allowing the morphogenetic gradient to change direction.
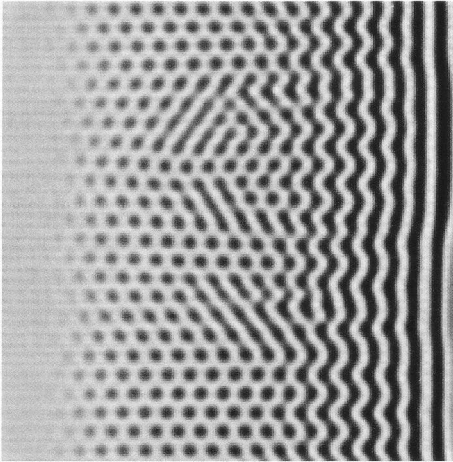
Fig. 14. Transition from an over-dispersed point pattern to a striped pattern using the Gray-Scott reaction-diffusion model (Pearson 1993) with convection in the x direction and a gradient in one parameter. $F = 0.04$, $k = 0.052$ (left) to $k = 0.059$ (right), $C = 0.02$. 200 by 200 grid, 20000 time steps.

Transitions between terrace lines and tubercles

If lateral inhibition was involved in the patterning of terrace lines and lirae, it is possible that the same regulatory networks were also involved in the spacing of tubercles and granules. As seen in Fig. 14, it is possible to move from a point pattern to a striped pattern by imposing a gradient in a single parameter of a reaction-diffusion system.

In *Paradoxides forchhammeri*, such transitions between terrace lines and granules are in fact observed, as shown in Fig. 15. In the transition zone, terrace lines seem to become shorter and more fragmented before they break up into granules, as would be expected in a system where terrace lines and granules are produced by a similar patterning system controlled by a gradient (see also Miller 1975: fig. 9). Moreover, it sometimes appears that granules are organized in rows. In pleurae, this seems to happen distally, towards the pleural spines, where the sculpturing changes to terrace lines. In the proximal region of the pleura, a special surface sculpture can even be observed which seems intermediate between terrace lines and granulation (Fig. 16).

Similarly, Bruton (1976) described rows of granules merging into raised lines (lirae) in *Phillipsinella preclara* (Middle Ordovician). The continuation of terrace lines into tubercles was also mentioned by Whittington (1997). These observations indicate that granules and terrace lines were to some extent controlled by the same underlying pattern formation system. Again, Notch mediated signalling is one possibility, as suggested for both random over dispersed patterns and striped arrangements of sensory bristles in dipterans (Simpson *et al.* 1999).

However, terrace lines are also seen in the earliest trilobites such as *Holmia*, and thus there is no known evolutionary sequence from point patterns to stripes as the one found in dipterans (Simpson *et al.* 1999). It is still of interest to note that granules and terrace lines are intergrading in relatively early trilobites such as *Paradoxides*. This

Fig. 15. Transition from an over-dispersed point pattern to a striped terrace line pattern. Partial lateral border/fixigena (mould) of *Paradoxides forchhammeri*, Middle Cambrian, Krekling, Norway (PMO164.580); × 2.3.
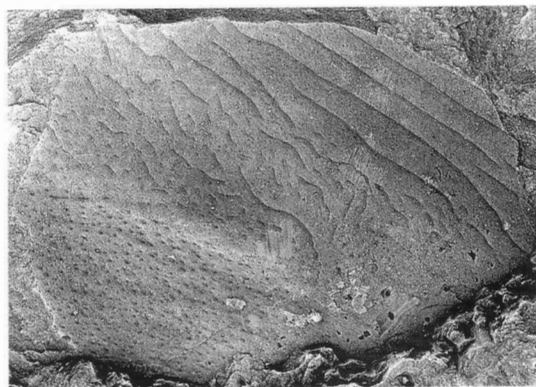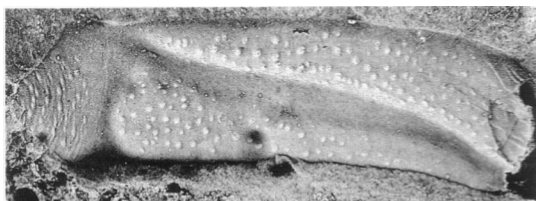

Fig. 16. Right pleura of *Paradoxides forchhammeri*, Middle Cambrian, Krekling, Norway (PMO164.579). Note the concentrically aligned tubercles proximally (left); × 2.7.

may indicate that the two structures had not yet diverged significantly in their developmental pathways within the trilobite lineage.

Conclusion

The observed overdispersion of trilobite tubercles and terrace lines is most easily explained with a lateral inhibition mechanism of the same type as known in other arthropods. In the Middle Cambrian trilobite *Paradoxides forchhammeri*, terrace lines and granules seem to be patterned in a continuum between lines and dots, indicating that the two structures share parts of a single underlying lateral inhibition system. It is impossible to speculate on whether the long-range inhibition mechanism was based upon diffusible factors, cell relay, mechanical processes or cell sorting, at least until this question has been resolved for living arthropods.

The lateral inhibition theory provides a conceptual framework for studying variation and evolution in trilobite cuticular patterning. In particular, it is unlikely that the trilobite genome coded for the positioning of each tubercle and terrace line individually. Rather, the cuticular pattern must be understood as the outcome of a morphogenetic process emerging from local interactions. Cuticular patterning can be useful for trilobite systematics (e.g., Tripp *et al.* 1977), but correct choice of characters necessitates some understanding of the developmental mechanism, at least at an abstract level. It has not been the goal of this paper to provide a formal theoretical morphospace (McGhee 1998), in part because the process is strongly dependent upon the compli-

cated boundary conditions imposed by the geometry of the exoskeleton. Still, the lateral inhibition theory provides two informal morphospace axes. The first is the distance between elements, determined by the range of lateral inhibition. The second is a continuous 'dots versus stripes' axis, determined by other parameters in the patterning system, as illustrated by computer modelling of abstract reaction-diffusion models. Similar continuity between dots and stripes can also exist under other paradigms of signal propagation.

Acknowledgments

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Organizacja przestrzenna guzków i linii pancerza trylobita *Paradoxides forchhammeri* świadczy o inhibicji bocznej

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Streszczenie

Analiza statystyczna rozmieszczenia guzków trylobita świadczy o istnieniu mechanizmu rozwojowego odpowiadającego za ich układ. Znany jest mechanizm inhibicji bocznej u owadów, odpowiedzialny za podobne odstępy szczecinek czuciowych, poznano także jego podłoże genetyczne. Guzki (granule) środkowokambryjskiego trylobita *Paradoxides forchhammeri* są rozmieszczone losowo, ale z zachowaniem podobnych odstępów. Zakładając, że rozmieszczenie obwodowych elementów nerwowych u wszystkich stawonogów opierało się na podobnym podłożu genetycznym, można pokusić się o przypuszczenie, że w ornamentacji pancerza trylobitów uczestniczyły geny o funkcjach zbliżonych do owadzich genów *Delta*, *Notch*, *achaete* i *scute*. U *P. forchhammeri* można także zaobserwować przejście między liniami tarasowymi (grzebieniami) a granulacją, co wskazywałoby na wspólny mechanizm determinacji tych struktur.