Regeneration in the anterior–posterior axis of the insect thoracic segment

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SUMMARY

After removal of a transverse strip of ventral thorax from the beetle, Tenebrio molitor, interaction occurred between epidermis posterior to the mesothoracic leg and that anterior to the metathoracic leg. Depending on the size and position of the excision, this interaction resulted in either the regeneration of the extirpated tissue or its replacement by an A/P reversed pattern of sclerites and supernumerary leg. By either route, local pattern continuity was restored between the normal meso- and metathoracic legs. Similarly, when a leg plus adjacent tissue was extirpated, continuity was restored by leg regeneration or by formation of an A/P reversed duplication of sclerites.

The results of these strip excisions can be understood in terms of two current models of the ventral thorax (the Boundary Model and the Polar Coordinate Model), each of which postulates a distinct compartment or region intervening between the epidermis surrounding the bases of successive legs. However, the models do not explain the large differences in the frequency of formation of the duplication/deletion pattern after excisions of different widths. The results are also compatible with a different model, involving an A–P sequence of positional values similar to that proposed for the abdominal segment. Regeneration would restore continuity within the sequence by the shortest route, forming either the midsegment (including the leg) or the intersegmental region.

The meso- and metathorax differ in the structure of the ventral sclerites and in the segmentation of the tarsus of the leg. The structures regenerated after the various excisions show that the segment border is not crossed during regeneration and indicate that an A/P compartment border running through the leg is usually also respected. There is no sign, however, of a third line of lineage restriction that would indicate a subdivision of the segment into three compartments (as proposed in the Boundary Model).

INTRODUCTION

Cells normally differentiate in ways appropriate to their position in the developing organism. In the early insect embryo, groups of cells in different positions along the longitudinal axis become segregated and develop into the different segments (see Sander, 1976). Within the segment precise cellular fate again depends on position and, in the postembryonic insect epidermis, this position is assessed locally, by interaction with adjacent cells. An operation confronting cells that are from different positions and hence have different positional values (Wolpert, 1971) is followed by intercalary regeneration which restores local

Key words: pattern formation, regeneration, insect segment, Tenebrio molitor, model, boundary, polar coordinate.
continuity by generating cells with intervening positional values (Bohn, 1970; French, 1978; Nübler-Jung, 1977; Wright & Lawrence, 1981a).

There have been several studies of the arrangement of positional values and lineage restrictions on parts of the two-dimensional epidermis of the thoracic segment. On the larval leg (and on the leg and wing imaginal discs of Drosophila) the results indicate that positional values change gradually down the proximal–distal axis and around the circumference of the appendage (French, Bryant & Bryant, 1976; Bryant, French & Bryant, 1981). Clonal analysis in Drosophila has shown that, from early embryonic stages onwards, the leg and wing primordia consist of anterior (A) and posterior (P) lineage compartments between which cells do not cross in normal development (Garcia-Bellido, Ripoll & Morata, 1973; Steiner, 1976). However, in the first stages of regeneration after damage cells can cross and contribute to the other compartment of the developing regenerate (Abbot, Karpen & Schubiger, 1981; Girton & Russell, 1980). There is some evidence that cockroach, cricket and beetle legs are also subdivided into anterior and posterior compartments but that these are still respected in intercalary regeneration following grafting experiments (French, 1980, 1984, 1986).

Relatively little work on the insect leg has been concerned with the relationship between the leg and the rest of the thoracic segment (the ventral sclerites, the lateral pleura and wing primordium, and the dorsal tergites). Bohn (1974) removed the leg and various parts of the cockroach ventral thorax, and analysed the conditions under which there was regeneration of the leg or duplication of the surrounding ventral sclerites. He showed that leg regeneration only occurred following interaction between thorax anterior and posterior to the leg base. He postulated three qualitatively different ‘zones’ (anterior, posterior and intersegmental) and proposed that a leg forms at an anterior/posterior junction. Meinhardt (1983, 1984) has interpreted Bohn’s and other results in terms of a basic subdivision of the embryonic segment into three compartments, with an appendage forming centred on the A/P boundary. A supernumerary appendage will regenerate if an extra A/P boundary is experimentally created.

In the present experiments we performed various strip excisions to confront different levels in the anterior–posterior axis of the ventral thoracic segment, to test the idea of subdivision of the segment into three distinct zones, and look for indications of lineage restrictions.

MATERIALS AND METHODS

Operations were performed on larvae of the beetle, Tenebrio molitor, which were reared at approximately 27°C and provided with wholemeal flour and damp cotton wool. Newly moulted larvae of the beetle, Tenebrio molitor, which were reared at approximately 27°C and provided with wholemeal flour and damp cotton wool. Newly moulted
larvae were kept for 1–2 days, anaesthetized under degassed water for approximately 2½ h, operated upon, kept overnight at 4°C to reduce movement and then reared separately in the wells of tissue culture dishes ('Multidish', Flow Laboratories) kept at 27°C. Operations involved removing precise strips of cuticle with underlying epidermis, extending from the ventral midline laterally to the edge of the dorsal tergite. Superficial cuts were made using knives made from chips of razor blade, any muscle attachments freed, the strip removed, leaking haemolymph
A/P regeneration in the insect thorax

Dried off and the anterior and posterior cut edges brought together and secured with insect wax (Krogh & Weis-Fogh, 1951), using a warmed needle.

Animals were selected by size to be in the penultimate larval instar; however, this was inaccurate as some metamorphosed at the next moult while others went through two further larval moults. Some of the latter were fixed as larvae but the rest were kept until the adult stage, partially dissected to remove internal organs and fixed in Carnoy or 100% ethanol. All animals were examined for external cuticular features and some were further dissected, digested in KOH and examined for the structure of leg sockets and internal cuticular sutures and apophyses. Sclerites from representative specimens were mounted in Gum Chloral for detailed examination of cuticular structures.

Fig. 2. Diagram of the adult ventral thorax with inset photographs (1–7) of cuticle preparations from particular regions. Anterior–posterior (A–P) and medial–lateral (M–L) axes are shown and leg bases are cross-hatched. The socket for the mesothoracic leg is formed partly from that segment (so) and partly from the metathorax (sop3), while the metathoracic leg socket is formed largely from the 1st (Abl) and 2nd abdominal segments, with some unsclerotized metathoracic cuticle (som) just behind the leg.

Anterior and lateral to the leg are large sclerites; the meso- and metasternum (A2 and A3), the meso- and meta-episternum (L2 and L3) and the meso- and metasternal epimeron (LP2 and LP3). Characteristic cuticular positional markers include long bristles on L2 and A2 (see insets 3 and 4) and on LP3 (see inset 7); tuft of long bristles (b) on L3 (inset 1); pits containing small bristles (e.g. region i of LP2, L3, A3 – polarity shown by arrows on insets 2, 5, 1, 6); dense spurs (e.g. region ii of LP2) and smooth cuticle. The smooth region iii of LP2 and the anterior region of L3 (shown by dashed lines) are normally folded under, out of sight. Prominent internal sutures between sclerites are shown by double lines, and the internal cuticular apophyses on the meso- (a2) and metathorax (a3) are shown in dotted lines. The spiracles (s2, s3, sAbl) are set in unsclerotized cuticle (dotted) in the anterior of the segment. Bars for all insets, 100 µm.
RESULTS

(A) The structure of the normal ventral thorax

The structures of the ventral meso- and metathorax of the larva and adult are illustrated in Figs 1, 2. The larval legs are largely surrounded by flexible, lightly sclerotized cuticle while the adult legs are deeply set in sockets and surrounded by large sclerites. As shown in Fig. 1C, these sclerites have characteristic shapes and characteristic cuticular features in several positions, and in many places the orientation of bristles marks anterior–posterior polarity. In both the meso- and metathoracic legs the trochanter articulates in the anterior face of the coxa, which bears a flange, while the posterior coxa is rounded with a suture. The legs differ in that the mesothoracic tarsus is subdivided into five and the metathoracic tarsus into four segments (French, 1986).

These various cuticular features allow the identification of the supernumerary legs and abnormal sclerite patterns produced after extirpation of parts of the larval segments.

(B) Removal of tissue posterior to the mesothoracic leg

Excision P1

Extirpation of a strip extending from immediately behind the mesothoracic leg approximately halfway to the metathoracic leg (see Fig. 3 for details) always resulted in regeneration of a normal pattern. Larvae examined after two moults appeared normal, except that parts of sclerites removed by the operation were not reliably replaced and the newly formed region was devoid of cuticular features. Adults formed after two or more moults were completely normal with respect to the structure of the legs, leg sockets and surrounding sclerites.

Excision P2

Extirpation of a larger strip extending posterior to the metathoracic spiracle (Fig. 3) led to two major classes of result; (i) regeneration of the normal pattern or (ii) in 60% of the animals, the formation of an abnormal pattern with a supernumerary leg inserted between the normal meso- and metathoracic legs. As in the previous experiment, the ‘normal’ larvae had not reliably replaced the extirpated sclerites and spiracle, but the adults were normal in all respects.

Of the 23 animals fixed as larvae, 9 formed a complete supernumerary leg which was usually positioned just behind the mesothoracic leg and was orientated with claw pointing medially. In particularly well-developed cases the asymmetry of the coxa and the curvature of the claw clearly showed that the leg was reversed in the anterior–posterior (A/P) axis.

In 23 of the 37 animals examined as adults a supernumerary leg was formed and, in all cases where its orientation could be determined, the limb was reversed in the A/P axis, with a (posterior) coxal suture facing anteriorly and an (anterior) coxal flange facing posteriorly (Fig. 4). The supernumerary coxa was usually adjacent to the mesothoracic coxa but was occasionally fused to it. From examination of
Fig. 3. Summary of the P, A and M series of excisions. The diagram shows the right side of the larval ventral meso- (T2) and metathorax (T3) with the anterior–posterior (A–P) and medial–lateral (M–L) axes shown. The leg bases (leg2, leg3) are crosshatched and the edges of the excisions are marked, extending from the ventral midline (M) to the edge of the dorsal tergite (t2, t3). The regions removed in each excision are shown by vertical bars. Bar, approximately 0.5 mm.

In the table of results, % refers to the percentage of surviving animals regenerating an abnormal deletion/duplication pattern (including those not forming a supernumerary leg). 4T/5T tarsi refers to the number of the scorable adult supernumeraries with tarsi which are anterior–metathoracic/posterior–mesothoracic (see text).

animals digested in KOH it was clear that the posterior part of the mesothoracic leg socket was missing (Fig. 4C). In all animals the supernumerary was separated from the metathoracic coxa by an abnormal sclerite which, from its cuticular
features and the partial reversal of polarity, was clearly a mirror-image duplication of the posterior part of sclerite A3 (see Fig. 5). The segmental composition of the supernumerary leg could be estimated from the structure of the tarsus in 14 cases, of which 11 were 4-segmented (metathoracic) on the anterior side and 5-segmented (mesothoracic) on the posterior side, with the 4-segmented part occupying more than half of the circumference (Fig. 4Bi). The three other animals had supernumerary tarsi which were 4-segmented only in a small region on the lateral side (Fig. 4Bii) or were entirely 4- or 5-segmented.

Medial to the supernumerary leg there was a duplication of the apophysis (Fig. 4C) and between the supernumerary leg and the wing bases the pattern of lateral sclerites was characteristically disrupted in the following way. The posterior part of sclerite LP2, the unsclerotized membrane containing the metathoracic spiracle and the anterior part of sclerite L3 were all missing, and were replaced by an A/P reversed duplication of the anterior part of LP2 and the posterior part of L3, separated by a suture (Fig. 5). In a further four adult animals there was a similar disruption of the lateral sclerites but no supernumerary leg was formed.

Excision P3

Complete removal of the tissue between meso- and metathoracic legs (Fig. 3) led to the regeneration of a supernumerary leg in 73% of animals, the rest forming a normal pattern. 13/21 larvae formed a supernumerary leg between the normal meso- and metathoracic legs (fused to the base of the metathoracic coxa in three cases), and in reversed A/P orientation.

Of the 25 animals fixed as adults, 20 formed a supernumerary leg which was usually adjacent to the mesothoracic coxa, with the posterior part of the mesothoracic leg socket missing (Fig. 6). In 19/20 cases the supernumerary was partially fused to the coxa of the metathoracic leg. The supernumerary was always reversed in the A/P axis and the segmentation of the tarsus indicated that it was usually (12/14 scorable cases) mesothoracic on its posterior side and metathoracic on its anterior side. In the remaining cases the mesothoracic (5-segmented) region occupied only a tiny part of the circumference. Medial to the supernumerary there was a duplication of the mesothoracic apophysis and usually a small symmetrical
piece of sclerite A3. In 18 cases the pattern of lateral sclerites could be scored. This was usually similar to that resulting from excision P2, except that all of sclerite L3 was missing and replaced by the duplication of the anterior part of sclerite LP2 (Fig. 6Bi), but in three cases the posterior part of LP2 was also present and was represented in the duplicate (Fig. 6Bii).

This series of experiments defines the most anterior level of the metathorax that can interact with tissue immediately behind the mesothoracic leg to give a supernumerary leg and a duplication of lateral sclerites.

(C) Removal of tissue anterior to the metathoracic leg

Excision A1

Removal of a strip extending from immediately in front of the metathoracic leg approximately halfway to the mesothoracic leg (Fig. 3) always resulted in regeneration of a normal pattern. As in other experiments, larval sclerites were not reliably replaced and the larval metathoracic spiracle was only sometimes reformed, but adult animals were completely normal.

Excision A2

Extrirpation of a larger strip extending anterior to the larval medial sclerites (Fig. 3) resulted in either the regeneration of a normal pattern or, in 49% of animals, in the formation of a supernumerary leg. Larval supernumeraries were usually partially fused to the metathoracic leg but well separated from the mesothoracic leg and were clearly reversed in the A/P axis.

In adults, the supernumerary leg was usually fused to the metathoracic coxa and appeared to be situated immediately behind the mesothoracic leg (Fig. 7A). However, examination of KOH-digested specimens showed that some of the posterior mesothoracic leg socket was present between mesothoracic and supernumerary coxae (Fig. 7C). Supernumerary legs were reversed in the A/P axis and the tarsal segmentation could be scored in eight cases, of which five were posterior–mesothoracic/anterior–metathoracic.

Medial to the supernumerary, the apophysis was duplicated and, laterally, sclerite L3 and the membrane containing the metathoracic spiracle were missing,

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Fig. 5. Results of excision P2.

(A) Whole-mount preparation of sclerites associated with formation of a supernumerary leg, showing duplication of parts of sclerites LP2, L3 and A3. Bar, 0.5 mm. Insets 1 and 2 show cuticular polarity (arrows) at the lines of symmetry in LP2 region i and in L3. Inset 2a is a higher power view of the same region of sclerite L3 (from a different specimen) showing pits and bristles with normal (n), intermediate (i) and reversed polarity (r). Bars on insets, 100 μm.

(B) Diagram of the typical result of excision P2. Heavy dashed lines mark the borders of the regenerated, A/P reversed region. Abbreviations and notations as in Figs 1, 3. (In 8/23 cases there was a more posterior line of symmetry on sclerites A3 and L3.)
but the complete sclerite LP2 was present and duplicated in A/P reversed orientation (Fig. 7B).

**Excision A3**

Extirpation of a similar strip extending a little further anteriorly into the mesothorax (Fig. 3) resulted in an increased frequency (82%) of formation of A/P reversed supernumerary legs. The structures formed in larval and adult animals were very similar to those resulting from excision A2, except that the posterior part of the adult mesothoracic leg socket was absent and the posterior part of LP2 was sometimes missing, as in excisions P2 and P3. The segmentation of the supernumerary tarsus could be scored in six cases, of which three were anterior–metathoracic/posterior–mesothoracic in structure.

This series defines the most posterior level of the mesothorax that can interact with tissue just anterior to the metathoracic leg to give a supernumerary leg and abnormal duplicated sclerite pattern. If the formation of a leg requires the interaction of anterior and posterior regions of the segment, it should occur following removal of the small strip (Bohn's 'intersegmental zone') separating these regions, but not from the extirpation of similar small strips from other positions.

Fig. 6. Results of excision P3.

(A) Ventral view of adult with A/P reversed supernumerary leg (Super) and a duplication of a small medial–posterior part of sclerite A3 (A3m).

(B) Whole-mount preparations of lateral sclerites showing (Bi) the typical result with a duplication of the anterior part of LP2 and (Bii) the minority result (3/18 scorable cases) with duplication of the entire LP2 (regions i, ii, iii). Bars, 0.5 mm.

(C) Diagram of the typical result of excision P3.
A/P regeneration in the insect thorax

(D) Removal of tissue midway between meso- and metathoracic legs

Excision M1

Extirpation of a small strip extending from just posterior to the metathoracic spiracle to just anterior to the medial sclerites (Fig. 3) resulted in the regeneration of a normal pattern in most animals. However, in three larvae and two adults (12% of animals) an A/P reversed supernumerary was formed (Fig. 8). Adult supernumerary legs were adjacent to the mesothoracic leg (although part of the posterior leg socket intervened) but separated from the metathoracic leg by a duplicated posterior sclerite A3. Lateral to the supernumerary, sclerite LP2 was complete and duplicated, as was the posterior part of sclerite L3. In two further adults there was a similar duplication of lateral sclerites but no supernumerary leg was formed.

Despite the low frequency of formation of supernumerary legs (see Discussion), the results of this experiment, plus the finding that extirpation of a strip of similar size from a more anterior position (excision M2 – see Fig. 3) always results in the regeneration of a normal pattern, are compatible with the interaction of specific anterior and posterior regions being required for leg regeneration. In this case the

![Diagram](image_url)

Fig. 7. Results of excision A2.

(A) Ventral view of adult with A/P reversed supernumerary leg. Abbreviations as in previous figures.

(B) Whole-mount preparation showing duplicated sclerite LP2 and the edge of the coxa of the supernumerary leg (Super). Bar, 0.5 mm. Inset 1 shows the duplication of LP2, regions i, ii, iii. Bar on inset, 100 μm.

(C) View from the inside (see Fig. 3C) showing the duplication of part of the posterior mesothoracic leg socket (sop), the supernumerary apophysis (Sa) and leg (Super) and the associated sclerites.

(D) Diagram of the typical result of excision A2, showing the A/P reversed regenerate (LP2, Super, Sa, A3m) between the heavy dashed lines.
removal of a leg plus a complete anterior or posterior region should lead to failure of leg regeneration.

(E) Removal of a leg plus adjacent tissue

Excision L

The removal of a transverse strip including the metathoracic leg always resulted in regeneration of the leg and normal sclerite pattern (see Fig. 9).

Excision LA

Removal of the metathoracic leg plus anterior tissue to a level just in front of the spiracle (Fig. 9) resulted in the regeneration of a leg and normal sclerite pattern in only 7/27 cases. In the other 20 adult animals the metathoracic leg was missing and so were most of the metathoracic structures normally anterior, lateral and medial to the leg, including sclerites \( A3, L3 \), most of \( LP3 \) and the metathoracic apophysis (Fig. 10). Intervening between the normal mesothorax and first abdominal segment was an expanse of fairly featureless unsclerotized cuticle (similar to that normally immediately posterior to a metathoracic leg) and, laterally, a partial sclerite which was perhaps a duplication of the posterior part of \( LP3 \). The adult

Fig. 8. Results of excision M1.

(A) Ventral view of adult, showing the A/P reversed supernumerary (Super) and the duplication of sclerite \( LP2 \) and parts of sclerites \( A3 \) and \( L3 \).

(B) Whole-mount preparation showing the duplicated sclerites \( LP2, L3, A3 \). Bar, 0.5 mm. Inset (i) shows polarity of pits and bristles (arrows) at the line of symmetry in sclerite \( L3 \). Bar on inset, 100 \( \mu \)m.

(C) Diagram of results of excision M1, with the A/P reversed regenerate between the heavy dashed lines.
Fig. 9. Summary of the L series of excisions. Abbreviations and notations as in Fig. 2. Bar, approximately 0.5 mm.

spiral was sometimes present (Fig. 10B), despite the larval spiral having been removed in the extirpation.

Excision LP

Removal of the mesothoracic leg plus posterior tissue to a level just in front of the metathoracic spiral (Fig. 9) gave regeneration of a normal pattern in only 3/17 cases. In the other 14 adult animals the mesothoracic leg was missing and so were many other mesothoracic structures, including most of the leg socket, the mesothoracic apophysis, the posterior part of sclerite A2, LP2 (in 13/14 cases) and the posterior part of L2 (in 11/14 cases). All metathoracic structures were present, including the spiral and the posterior rim of the socket of the mesothoracic leg. Intervening between the anterior edge of the metathorax and the normal anterior structures of the mesothorax was an A/P reversed duplication of anterior sclerites which was variable in structure and usually partially metathoracic (e.g. the rim of the leg socket and the anterior part of A3) and partially mesothoracic (e.g. the

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Fig. 10. Results of excision LA.

(A) Ventral view of adult showing the absence of metathoracic leg (leg3) on the operated side. Intervening between the mesothorax and the abdominally derived part of the leg socket (Ab1, Ab2) is an expanse of unsclerotized cuticle (m), the posterior part of LP3 and an additional partial sclerite (Scl).

(B) Camera lucida drawing of specimen dissected to illustrate the presence of a complete LP2 (regions i, ii, iii) and part of the posterior mesothoracic leg socket (sop). As in 6/22 cases, a small metathoracic spiracle (s3) is present.

(C) Diagram of the result of excision LA with the expanse of membrane interpreted as a duplication of posterior metathoracic membrane (som) and the additional sclerite as a duplication of the posterior part of LP3.
Fig. 11. Results of excision LP.

Ventral views of adult thoraces showing replacement of the mesothoracic leg and posterior sclerites by an A/P reversed duplication of anterior sclerites. A–C illustrate the variation in the structure of the duplication.

(A) Largely mesothoracic duplicate consisting of large parts of L2 and A2, with small regions of anterior A3 and the metathoracically derived part of the leg socket (sop3). There is a normal metathorax with spiracle (s3) and wing base (W3), and a supernumerary wing base (WS) between the meta- and mesothoracic (W2) wings. Bar, 0.5 mm. Insets ① and ② show cuticular polarity at the line of symmetry in sclerites L2 and A2. Bars on insets, 100 μm.

(B) Largely metathoracic duplicate consisting of part of the leg socket (sop3), and large parts of sclerites A3 and L3 (including the tuft of bristles – b), with a small region of A/P reversed A2. Bar, 0.5 mm. Inset ③ shows the junction of the reversed A3 and L3 duplicate with the normal A2 and L2 (polarity indicated by arrows). Bar on inset, 100 μm.

(C) Diagram of a duplication of metathoracic part of the socket (sop3) and a sliver of sclerite A3, plus the anterior membrane (am) and anterior parts of sclerites A2 and L2 of the mesothorax.
anterior parts of sclerites \( A2 \) and \( L2 \), as shown in Fig. 11. The very lateral edge of the duplication was difficult to score and usually damaged in the preparation of the specimens, but in two cases there was a clear supernumerary wing base (Fig. 11B). This aspect of the results is under further study.

The results of this series of experiments again indicate that the formation of a leg requires the interaction of tissue anterior and posterior to the leg base. If one of these regions is removed together with the leg, regeneration restores continuity in the anterior/posterior axis by forming a duplication of sclerites.

**DISCUSSION**

(A) **Correspondence between the larval and adult segment**

The larval and adult thoracic segments of *Tenebrio* are very different in structure and it is not known precisely which parts of the larval segment give rise to the adult structures. Huet & Lenoir-Rousseaux (1976) showed that the larval leg develops into the adult leg, and this was supported by grafting experiments (French, 1986) where a graft/host junction at the base of the larval leg generated adult supernumerary legs at the level of the proximal or mid coxa. In most of the present experiments, the extirpation of transverse strips from the larval ventral thorax causes parts of the adult ventral thorax to be absent and replaced by duplications.
in reversed A/P orientation. The positions of the edges of the larval excisions are related in a regular way to the positions of the edges of the pattern disruptions in the resulting adults. This suggests a correspondence between larval and adult segments (Fig. 12) in which larval tissue anterior to the leg develops into adult anterior sclerites, the ‘intersegmental membrane’ and the spiracle, while tissue posterior to the leg makes part of the leg socket. The most posterior part of the socket of the mesothoracic leg is made by the anterior of the larval metathorax, and the most posterior part of the large metathoracic leg socket develops from the 1st and 2nd abdominal segments. The adult wings have been shown to develop from the lateral edges of the larval meso- and metathoracic tergites (Hundertmark, 1935), as illustrated in Fig. 12.

(B) Regeneration between different A/P levels of the segment

If a transverse strip of larval thorax is removed from the intersegmental region, as in the A, P and M series of experiments, the tissue posterior to the mesothoracic leg can interact with that anterior to the metathoracic leg. As first shown by Bohn (1974) on the cockroach, this can result in either the regeneration of the extirpated intersegmental region (restoring the normal pattern), or its replacement by an A/P reversed duplication of sclerites and a supernumerary leg. Exactly which parts of the sclerites and leg socket are duplicated depends on the position of the extirpation (Fig. 12) and the effect is to restore continuity to the pattern between the meso- and metathoracic legs and, more laterally, between the legs and the wing bases (e.g. Fig. 5). Similarly, when one of the legs plus adjacent tissue is removed (the L series of excisions) regeneration restores pattern continuity, either by replacing the extirpated tissue or by inserting an A/P reversed duplication of parts of the socket and sclerites (see also Bohn, 1974).

In those cases where regeneration results in a deletion/duplication of pattern elements, continuity is restored in the A/P axis of the animal but, of course, continuity cannot be established between the A/P reversed regenerate and the unoperated tissue on the other side of the ventral midline, or that lateral and dorsal to the regenerate. In the region of the ventral midline there is usually a ridging or distortion of the cuticle (see Fig. 4) and sometimes the persistence of an unhealed region. The lateral region, comprising membrane and wing axillary sclerites, is difficult to score and was usually damaged in preparing the specimens.

Only some excisions produce the deletion/duplication pattern, while others are always followed by replacement of the excised parts. Bohn (1974) suggested that a leg was only replaced, or a reversed supernumerary leg regenerated, when the excision confronted tissue from two ‘zones’, respectively anterior and posterior to the leg base. In the normal pattern, zones from successive segments are separated by a third, qualitatively different zone. More recently, Meinhardt (1983, 1984) has extended Bohn’s ideas, proposing that the segment consists of three transverse ‘compartments’ (S, A and P) at least one of which is further subdivided into medial and lateral. The leg is normally formed at the intersection of A/P and M/L
Fig. 12. Correspondence between the adult (A) and larval (B) ventral thoracic structures of Tenebrio, as suggested by the extent of adult deletion/duplication patterns resulting from the various larval excisions. The leg bases are cross-hatched, and the adult wing bases (W2 and W3) and larval wing primordia are filled in.
boundaries, and a supernumerary leg forms if the S region is removed, creating a new P/A boundary (Fig. 13A).

Bohn's results and the present Tenebrio results can also be interpreted in terms of the Polar Coordinate Model (French et al. 1976) in which the leg epidermis contains a map or field of circumferential and proximal/distal positional values which extends back onto a surrounding ring of thoracic epidermis. Removal of the intersegmental area (which is not covered by the limb fields) allows intercalary regeneration between cells with different values in the successive fields, and results in the formation of an extra circumference of values and hence in a supernumerary leg (see French, 1980, for details).

In both of these models, a qualitative difference exists between the thoracic tissue adjacent to the leg base and the intervening intersegmental band. In principle, a supernumerary leg should always form if this band is completely removed, but in the present experiments even the removal of almost all tissue between successive leg bases (excisions A3, P3) gave this result in only 70–80% of cases. Similarly, Bohn (1974 – expts C5, C6) obtained supernumeraries in only 40–60% of cases. These large excisions may heal irregularly and it is possible that interaction sometimes occurred, not between A and P edges as intended, but between M and L edges, and was followed by regeneration in that axis. This is less likely to have been the case, however, in the present work, where the A and P edges were held together with wax.

In these experiments we attempted to test the idea of a distinct intersegmental band. We defined the anterior limit of the metathoracic leg field by a series of broad extirpations starting just behind the mesothoracic leg (P series), and the posterior limit of the mesothoracic leg field by extirpations starting just in front of

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Fig. 13. Two models of the larval ventral thorax. The extent of various strip excisions is indicated by vertical bars.

(A) The Boundary Model in which the thoracic segment is divided into three transverse compartments (S, A, P) with at least the A compartment subdivided into M and L. Legs (leg2, leg3) are formed at the A/P compartment borders, so a supernumerary leg will be regenerated in reversed orientation after excisions P2 and M1, but not after P1 (which confronts compartments P and S). Similarly, removal of the leg plus the entire P compartment (excision LP) confronts A and S, so the leg is not regenerated.

(B) An A-P Sequence Model, involving positional values (0–10) down the A-P axis. An excision creates a discontinuity in this sequence and is resolved by intercalary regeneration by the shortest route. A difference of five values gives two equally probable results, while a difference of four gives a majority and a minority result. Excision P1 confronts 7 and 10/0 and will regenerate the normal pattern (8, 9), while excision P2 confronts 7 and 2 and regenerates either 8, 9, 10/0, 1 or 6, 5, 4, 3 (corresponding to sclerite duplication and a supernumerary leg). M1 confronts 8 and 2 and usually regenerates via 9, 10/0, 1 but occasionally via 7, 6, 5, 4, 3. Excision LP confronts 3 and 10/0 and will regenerate via 2, 1, forming a duplication of anterior sclerites. In this form the model explains most of the results, including the differences in frequency. One anomaly is the L excision which, confronting 3 and 10, is expected (but not observed) to give a minority result in which the leg is replaced by duplicated sclerites.
the metathoracic leg (A series). The models outlined above would predict that a narrow excision that removed the tissue between these limits would produce a supernumerary leg. However, while this extirpation (M1) did indeed give supernumeraries, the frequency was very low (12 %, compared to 49 % and 60 % from the broad excisions which defined the limits) and, in a similar experiment, Bohn (1974 – expt C7) failed to get any supernumeraries. The narrow excisions might be expected to heal more reliably and hence give a higher rather than a much lower frequency of supernumeraries. These unexpected results suggest that the size as well as the position of an excision is important.

The influence of excision size suggests an alternative, quantitative model of the A/P axis of the thoracic segment, resembling that previously proposed for the abdomen. Grafting experiments on a range of insects indicate that the abdominal segment contains an A–P gradient of positional values (reviewed by Lawrence, 1973b). In an elegant series of strip extirpations and strip grafts on the bug, Oncopeltus, Wright & Lawrence (1981a) extended this model. They showed that interaction between cells differing in position by half a segment or less resulted in regeneration of the missing tissue, while a greater discrepancy was resolved by formation of a deletion/duplication. Hence a reversed ectopic segment border was formed after a broad excision confronting very anterior and posterior levels of the segment. They proposed that intercalary regeneration occurs by the shortest route within the segmentally repeating gradient or sequence of positional values. In a similar model for the thoracic segment the ‘intersegmental membrane’, sclerites, socket and leg would all be formed in response to different positional values in a segmentally repeating A–P sequence within which discontinuities are resolved by the shortest route (Fig. 13B). It is clear from the results that the position of the excision as well as its size are important (since excisions M2, A1 and P1 never gave supernumeraries) so the positional values must be unequally spaced and clustered in the regions of the leg base and the segment border. This model could account for the different frequencies of duplication/deletion from the different strip excisions (Fig. 13B), but would require a separate system to give medial–lateral positional information and govern regeneration in that axis (Rowlands & French, in preparation).

None of these models gives a complete representation of the insect ventral thorax. The A–P Sequence Model (Fig. 13B) requires a different arrangement of positional values on the leg itself, since the pattern of intercalation on the leg argues strongly for polar rather than Cartesian (A–P and M–L) axes (French, 1981), while the Polar Coordinate Model requires an additional system of positional information in the intersegmental region. The Boundary Model (Fig. 13A) gives a coarse subdivision into three bands and requires additional specification of position within these bands. Also, although the models can explain the number, orientation and position of supernumerary legs formed when the larval Tenebrio leg is grafted to the contralateral side, they cannot account for the results of a 180° rotation (French, 1986).
Most of the present strip excisions result in the confrontation of different levels of the meso- and metathorax, and, due to the different adult cuticular structures of these segments, the contribution made by each cut edge to the regenerate can be estimated. In cases where the pattern is restored (i.e. where the intersegmental region is formed in the A, P and M series, or the leg in the L series), the pattern is completely normal. This shows that both edges contribute and, furthermore, that their contributions stop at the segment border. Otherwise, for instance, the regenerated anterior metathorax would sometimes bear some ectopic anterior mesothoracic structures.

In cases where a duplication/deletion pattern is formed, the cuticular structures again indicate that both edges of the excision participate, but the extents of their contributions are more variable. Examination of the tarsi of the supernumerary legs formed in the A, P and M series shows that, in all experiments, the majority of legs had metathoracic structure (4 segments) on their anterior circumference and mesothoracic structure (5 segments) on a smaller posterior region. However, in each experiment, a minority of supernumeraries differed from this in the extent or location of the regions. When the *Tenebrio* prothoracic leg is grafted A/P reversed into the metathoracic leg site, supernumeraries are formed in A and P positions. They are reliably half-and-half in composition with the borders just posterior to midlateral and midmedial (French, 1986), as in the majority of the present supernumerary legs. Similar results are found at levels within the *Tenebrio* and cricket leg (French, 1984), and suggest that the larval leg is divided into A and P lineage compartments which are respected during regeneration. The present results give some support to this notion, although it seems that the border can sometimes be crossed when regeneration commences from a level on the thorax.

The structure of the lateral sclerites formed after the various extirpations is also consistent with a lineage restriction within the segment. In excisions P2 and M1 parts of both meso- and metathoracic sclerites are formed, with the border falling reliably between the anterior edge of *LP2* and the posterior edge of *L3* (Figs 4, 7). This suggests that there may be anterior–posterior compartment borders between sclerites *L2* and *LP2*, and between *L3* and *LP3*. In excisions P3, A3 and A2, however, it seems that only all or part of mesothoracic sclerite *LP2* is formed, perhaps because the metathoracic edge of these excisions is more posterior and, being nearer to the compartment border, can regenerate little or no more posterior tissue.

The results of the strip excisions are generally compatible with the three ‘compartment’ Boundary Model (Fig. 13A) although, as explained in the previous section, this does not explain the large differences in the frequency of classes of result. In the model the segment border corresponds to the boundary between the P and S ‘compartments’, while the boundary between A and P runs through the leg base. The structure of the regenerated legs and sclerites indicates that the segment (P/S) border is not crossed during intercalary regeneration, and is consistent with
an A/P compartment border which is usually also respected. There is no indication, however, of a third (S/A) lineage border being respected during intercalary regeneration. In the critical experiment, LP, interaction between A mesothoracic and S metathoracic 'compartments' reliably produced a duplication of anterior segmental structures, but the junction between the meso- and metathoracic components does not fall in a constant position (Fig. 11). These experiments, then, provide no evidence for subdivision of the thoracic segment into three, as opposed to two, lineage compartments.

There have been several studies of cell lineage and regeneration in the abdominal segment of *Oncopeltus* (Lawrence, 1973a; Wright & Lawrence, 1981a,b) but at present it is not clear from clonal analysis whether there is subdivision into two or three compartments (D. Wright, personal communication). By grafting between colour mutants, Wright & Lawrence (1981b) showed that cells of the abdominal segment will intercalate up to but not across the segment border, but it has not been shown whether the cells observe any other lineage restriction when regenerating in response to grafting experiments.

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A/P regeneration in the insect thorax


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