

# Interaction between the leg and surrounding thorax in the beetle

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## SUMMARY

Interactions between the insect leg and surrounding thoracic epidermis were studied in the beetle, *Tenebrio*, by grafting the entire larval prothoracic leg into the metathoracic leg site in various orientations.

Control grafts simply heal, but A/P reversed grafts lead to regeneration of supernumerary legs of host orientation in A and P positions. M/L reversed grafts also give supernumeraries, again with host orientation but in M (or MP) and L (or LA) positions. The differences in structure between adult prothoracic and metathoracic legs allow the origin of these supernumeraries to be analysed at coxa and tarsus level. The A/P supernumeraries are *consistent* and *complementary* in structure, of host origin on the host side and graft origin on the graft side, and with the borders in apparently constant midmedial and midlateral positions. The M/L supernumeraries, however, are *variable* and often *non-complementary* in structure. The results of the A/P and M/L reversals are similar to those found at a more distal level in *Tenebrio* legs and the legs of several hemimetabolous insects, suggesting that the arrangement of positional values and A and P compartments extends from the epidermis of the leg onto the surrounding thorax.

The results of a 180° rotation of the entire leg, however, differ from those found at a more distal level in that the grafted leg rarely derotated and two (or occasionally one or three) supernumeraries are formed in a wide variety of positions, some with constant and others with variable orientation. These results are not readily explained by current models of insect leg formation and regeneration.

## INTRODUCTION

The cellular interactions underlying pattern formation in the insect epidermis have been studied in numerous extirpation and grafting studies on the abdominal segments and thoracic appendages of a variety of hemimetabolous insects (and some holometabola such as *Drosophila*). This work suggests that postembryonic epidermal cells have stable 'positional values' (Wolpert, 1971) which determine which cuticular structures are formed, while the relationship between values of neighbouring cells determines the polarity of structures and perhaps also the degree of local cell division.

The results of grafting experiments indicate that cells within the abdominal body segment gradually differ in positional value along the anterior–posterior axis and

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that this 'gradient' is repeated in successive segments (reviewed by Lawrence, 1973). Grafting and extirpation experiments on larval insect legs (and on *Drosophila* imaginal discs) have suggested a two-dimensional map of positional values, arranged along the proximal-distal axis and around the circumference of the appendages (French, Bryant & Bryant, 1976). In both legs and abdominal segments a graft which confronts normally non-adjacent cells is followed by *intercalary regeneration*, producing cells with the shortest set of intermediate values (Bohn, 1970; French, 1978; Nubler-Jung, 1977; Wright & Lawrence, 1981), thereby removing the discontinuity in positional values.

It has been shown in cockroaches (Bohn, 1974) and in beetles (Lender & Grobocopateli, 1967) that the complete removal of the larval leg results in its regeneration from the surrounding thoracic body segment, and Bohn (1974) has shown that this depends on interaction between thorax anterior and posterior to the leg base. As a first step in the investigation of the relationship between the arrangement of positional values on the leg epidermis and on the surrounding thorax of the beetle, *Tenebrio*, the present experiments study interaction between thorax and the base of a grafted leg which has one transverse axis reversed (contralateral graft) or has been rotated by 90° or 180° (ipsilateral graft).

Numerous contralateral and ipsilateral grafting experiments have been done at coxa or tibia level *within* the larval legs of cockroaches (Bohn, 1965, 1972; Bulliere, 1970), stick insects (Bart, 1971) and crickets (French, 1984), and they frequently result in the regeneration of supernumerary branches from the graft/host junction. After reversal of the anterior-posterior (A/P) axis of the graft, two supernumerary distal regenerates form, usually in anterior and posterior positions and with host handedness and orientation, but sometimes fused together and in medial or lateral position. After medial-lateral (M/L) axis reversal, the two supernumeraries form medially and laterally, or are fused together and in anterior or posterior position. Each supernumerary is usually composed of both graft and host-derived tissue (Bohn, 1972) and, at least in the case of cricket legs, the A/P supernumeraries are reliably half-and-half in origin (French, 1984). After a 90° rotation, the graft derotates back into alignment with the host and no supernumeraries are formed (Bohn, 1965; Bulliere, 1970). After a 180° rotation, the results are very variable, between individuals and between species. The graft usually derotates and sometimes no supernumeraries are formed. Sometimes two supernumeraries, one of host- and the other of graft-handedness, are formed in various positions and orientations, and often there is only one symmetrical supernumerary which is often a small distally incomplete structure (Bohn, 1965, 1972; Bulliere, 1970). In the stick insect, three supernumeraries are sometimes formed (Bart, 1971).

Here I show that a similar pattern of supernumerary legs is formed after a range of grafting operations changing the relationship between the *Tenebrio* leg and surrounding thorax (although with differences in the case of the 180° rotation), suggesting a similar arrangement of positional values on the leg and over the surrounding thoracic segment.

## MATERIALS AND METHODS

Experiments were performed on two species of the beetle, *Tenebrio* (*T. molitor* and *T. obscurus*), which were reared in plastic containers at approximately 27°C and provided with wholemeal flour and damp cotton wool. Adults were kept separately from developing larvae and pupae, and newly moulted larvae were selected and kept for 2–3 days before the operation. An attempt was made to use animals in their penultimate larval instar, but it is difficult to select reliably on the basis of size, so some operated larvae moulted directly to pupae and others went through two further larval instars before metamorphosis. The two species were used because initially it seemed that the slight colour difference would provide a useful cuticular marker. However, although interspecies grafts are readily accepted, the colour difference is not adequate for this purpose. Results from the two species are very similar and will be discussed together.

Experimental animals were anaesthetized under degassed water for approximately 3 h, amputated or grafted using fine forceps and knives made from chips of razor blade (Gillette Francaise), kept overnight at approximately 4°C to reduce movement and resulting loss of unhealed grafts, and then kept in small numbers in lunch boxes at 27°C. A few operated animals which went through two larval moults were then fixed as larvae, but the others were kept until the adult stage, fixed in Carnoy and examined.

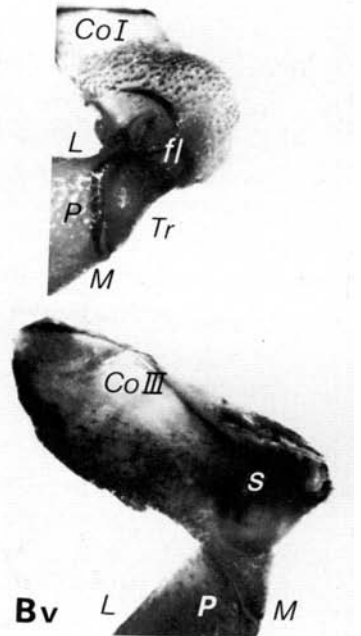
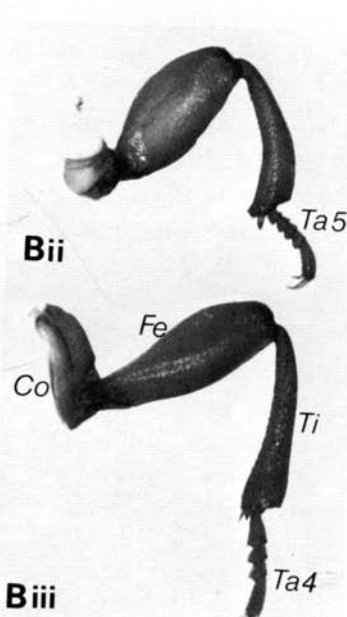
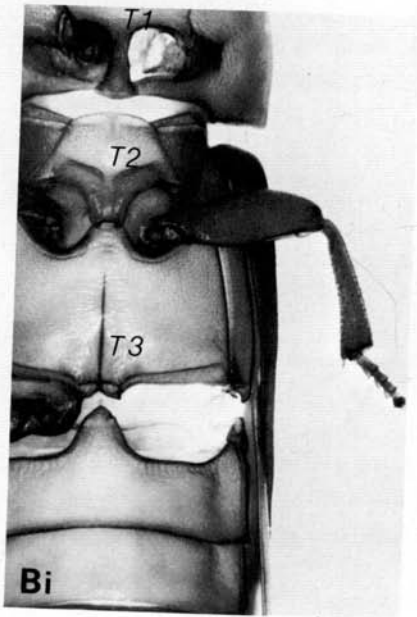
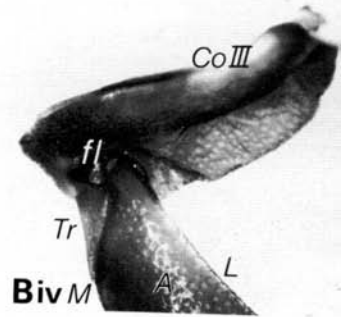
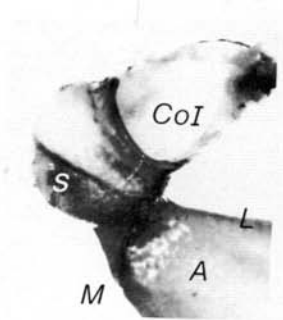
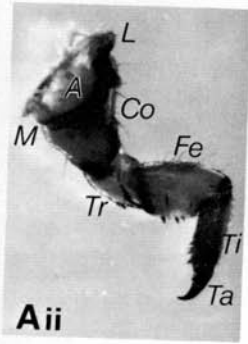
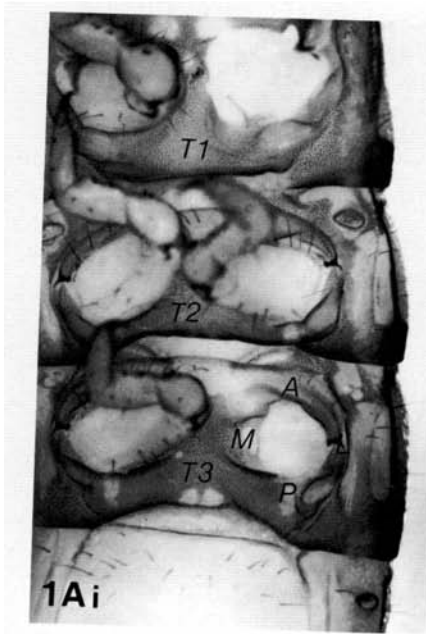
## RESULTS

### (A) *The structure of the larval and adult legs*

The structure of the ventral thorax and the prothoracic and metathoracic legs of larval and adult *Tenebrio* is shown in Fig. 1. The legs of *T. obscurus* are slightly larger (and slightly darker in colour) than those of *T. molitor* but the cuticular structures are almost identical. Grafting experiments (see below) were performed between prothoracic and metathoracic legs since the adult legs differ somewhat in structure and this serves as a marker for graft or host origin of regenerated structures.

On the adult prothoracic leg (Fig. 1Bii), the anterior face of the coxa is rounded with an obvious suture, while the posterior face has a prominent flange and articulates with the trochanter. The two large spines on the distal tibia are directed slightly posteriorly and the tarsus is subdivided into five segments. When the larval leg plus a small ring of surrounding thorax is removed (as in the grafts of Section B) the remaining tissue can regenerate a leg. If the animal moults into a larva, a small larval leg is produced and this lacks many of the normal spines and long hairs. However these animals, and those moulting directly into pupae, develop a normal adult leg socket and a leg with the typical coxa/trochanter articulation and the five-segmented tarsus (12/15 cases: the others were poorly segmented).

On the adult metathoracic leg (Fig. 1Biii), the trochanter articulates in the anterior face of the coxa, which bears a flange, while the posterior coxa is rounded with a suture. The two distal tibial spines are directed slightly anteriorly and the tarsus consists of only four segments. When the larval leg is removed at the base, the surrounding ventral thorax can regenerate a small larval leg which has a variable pattern of spines and hairs. Operated animals develop a normal adult leg



with typical coxa/trochanter articulation and the four-segmented tarsus (21/28 cases: the others were broken or poorly developed).

(B) *Grafts of the leg to the leg site*

The left metathoracic leg of the host was amputated around the base and the donor left (series i, iv, v) or right (series ii, iii) prothoracic leg plus a small ring of surrounding thorax was removed and grafted with appropriate orientation to the host leg site. The ring of thorax was pushed beneath the rim of the host site to secure the graft.

(i) *Control orientation*

The left prothoracic leg was grafted without rotation to the left metathoracic leg site (Fig. 2A) and in animals subsequently moulting into larvae the graft had healed in normal orientation, with no sign of supernumerary structures.

In all 49 successful cases which developed into adults there were no supernumeraries and the coxa of the grafted leg was clearly of prothoracic structure. The more distal parts were sometimes broken (13 cases) or poorly segmented (7 cases) but in 29/49 cases they were clearly prothoracic, with tibial spines directed posteriorly and a five-segmented tarsus (Fig. 2B). Proximal to the leg, the sclerites and leg socket were usually normal metathoracic structures, but occasionally the rim of the leg socket resembled that of the prothorax.

(ii) *Anterior-posterior axis reversal*

The right prothoracic leg was grafted into the left metathoracic leg site with reversal of the A/P axis (Fig. 3A). In animals subsequently moulting into larvae, the graft retained its orientation and was usually accompanied by bulges or distinct supernumerary legs anterior and/or posterior to the graft.

77 animals developed to adults with a grafted prothoracic leg which was, in all cases, orientated with its A/P axis reversed relative to the host and was accompanied by supernumerary leg structures. In 61/77 cases separate supernumeraries were formed in anterior (47/61) or anterior medial (14/61) and in posterior (40/61) or posterior medial (21/61) positions and were orientated like the host, claws pointing medially (Fig. 3B). In some cases the coxae of the supernumeraries were completely separated from that of the graft but usually there was one large

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Fig. 1. Structure of the ventral thorax and legs of larval and adult *Tenebrio molitor*. (A) Larval structures. Ventral view of thorax (i) after removal of prothoracic leg plus ring of surrounding tissue (shown separately in ii) and metathoracic leg. *T1*, *T2*, *T3*, pro-, meso- and metathoracic segments; *A*, *P*, *M*, *L*, anterior, posterior, medial and lateral; *Co*, *Tr*, *Fe*, *Ti*, *Ta*, coxa, trochanter, femur, tibia and tarsus. (B) Adult structures. Ventral view of thorax (i) after removal of prothoracic leg (shown in ii) and metathoracic leg (shown in iii). Note the five-segmented prothoracic tarsus (*Ta5*) and the four-segmented metathoracic tarsus (*Ta4*). Enlarged anterior (iv) and posterior (v) views of the prothoracic coxa (*CoI*) and metathoracic coxa (*CoIII*) show the differences in location of the suture (*s*) and flange (*fl*).

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coxal base separating into three apices. Proximal to the legs, the sclerites and leg socket were usually normal metathoracic structures.

The supernumeraries were sometimes poorly developed and often their distal parts were damaged, but in those cases where their structure could be analysed it was remarkably *consistent* (Fig. 4). The *anterior* supernumerary coxal apex could be analysed in 54 cases and always had a flange (characteristic of anterior metathoracic coxa) on the anterior face and another flange (found on posterior prothoracic coxa) on the posterior face. The tarsus was broken in 34 cases, rudimentary in 18 cases, but in the 9 analysable cases there were four segments on the anterior side and five on the posterior side. The *posterior* supernumerary coxal apex could be analysed in 55 cases and always had a suture but no flange (characteristic of anterior prothoracic and posterior metathoracic coxa) on both anterior and posterior faces. The tarsus was broken in 28 cases, rudimentary in 16 cases, but in all 17 analysable cases there were five segments on the anterior and four on the posterior side. Thus all supernumeraries which could be analysed at coxa or tarsus level had host (metathoracic) structures on the host side and graft (prothoracic) structures on the graft side. The two supernumeraries formed on a grafted leg were strictly complementary in structure.

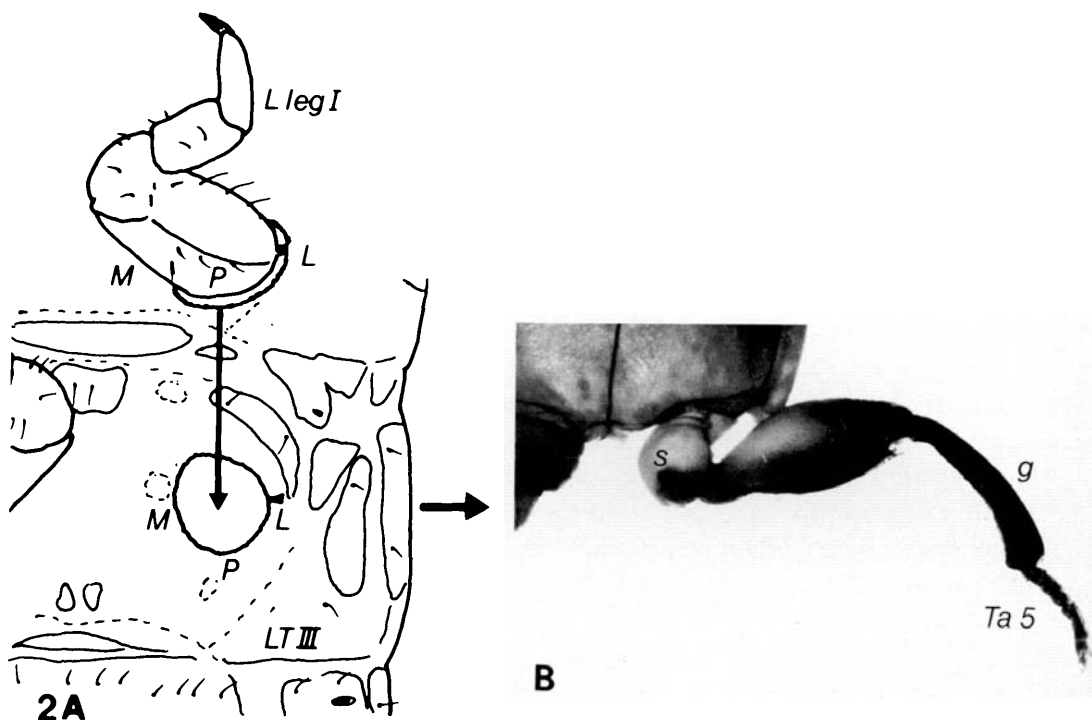


Fig. 2. Leg-to-leg site: control graft. (A) Schematic view of the operation, grafting the larval left prothoracic leg (*Lleg I*) into the left metathoracic leg site (*LT III*). (B) Anterior view of resulting adult metathorax (abdomen removed) showing grafted prothoracic leg (*g*) with five-segmented tarsus (*Ta5*) and anterior coxal suture (*s*).

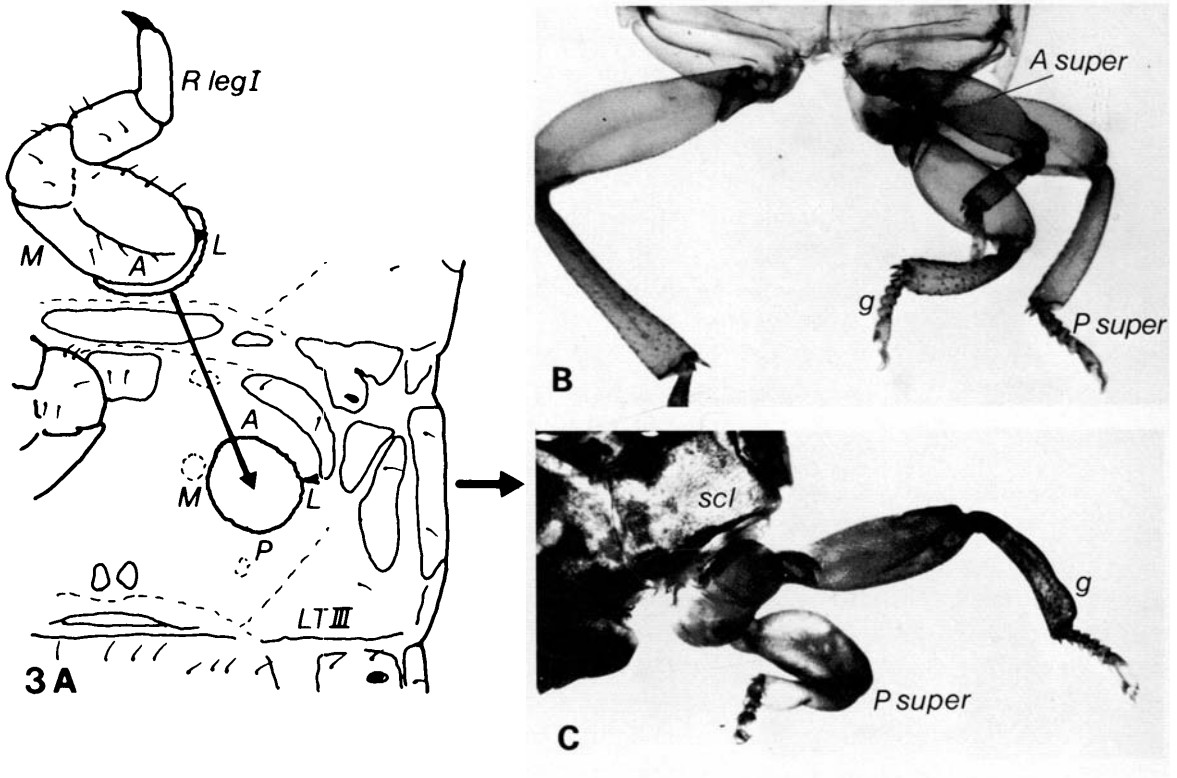


Fig. 3. Leg-to-leg site: anterior–posterior axis reversal. (A) Schematic view of the operation, grafting the right prothoracic leg (*R leg I*) into the left metathoracic leg site (*LT III*), reversing the A/P axis. (B) Anterior view of resulting adult thorax showing the majority result with the grafted leg (*g*) plus anterior (*A super*) and posterior (*P super*) supernumerary legs. (C) Anterior view of minority result with the grafted leg, a posterior supernumerary and, anterior to the graft, a small duplicated region of thoracic sclerite (*scl*) and no supernumerary.

In a further 3/77 cases there was extensive fusion between the graft and one or both of the supernumerary legs. The remaining 13/77 had lost most or all of the sclerite normally anterior to the leg and bore just the A/P reversed graft plus a posterior supernumerary which was half-and-half in structure, like the posterior supernumerary legs described above (Fig. 3C).

### (iii) Medial–lateral axis reversal

The right prothoracic leg was grafted with reversal of the M/L axis into the left metathoracic leg site (Fig. 5A). In animals subsequently moulting into larvae the graft retained its orientation and was accompanied by bulges or supernumerary legs in approximately lateral and medial positions.

In 59/68 successfully grafted animals which developed to adult, the grafted prothoracic leg was orientated with its M/L axis reversed relative to the host, and

was accompanied by supernumerary legs in lateral (30/59) or lateral–anterior (29/59) and in medial (41/59) or medial–posterior (18/59) positions. The supernumeraries were all orientated approximately like the host, with claws pointing medially (Fig. 5B). As in the previous graft series, the grafted and supernumerary legs separated either at the base of the coxa or slightly more distally, and the thoracic sclerites and leg socket were usually metathoracic in structure. In contrast to the supernumeraries formed after A/P reversal however, these M/L supernumeraries were *variable* in structure in the two regions (coxal apex and tarsus) where graft- and host-type structures could be distinguished. In many cases the graft (and host) tissue did *not* form complementary structures on the two supernumeraries of a given leg (Fig. 5C).

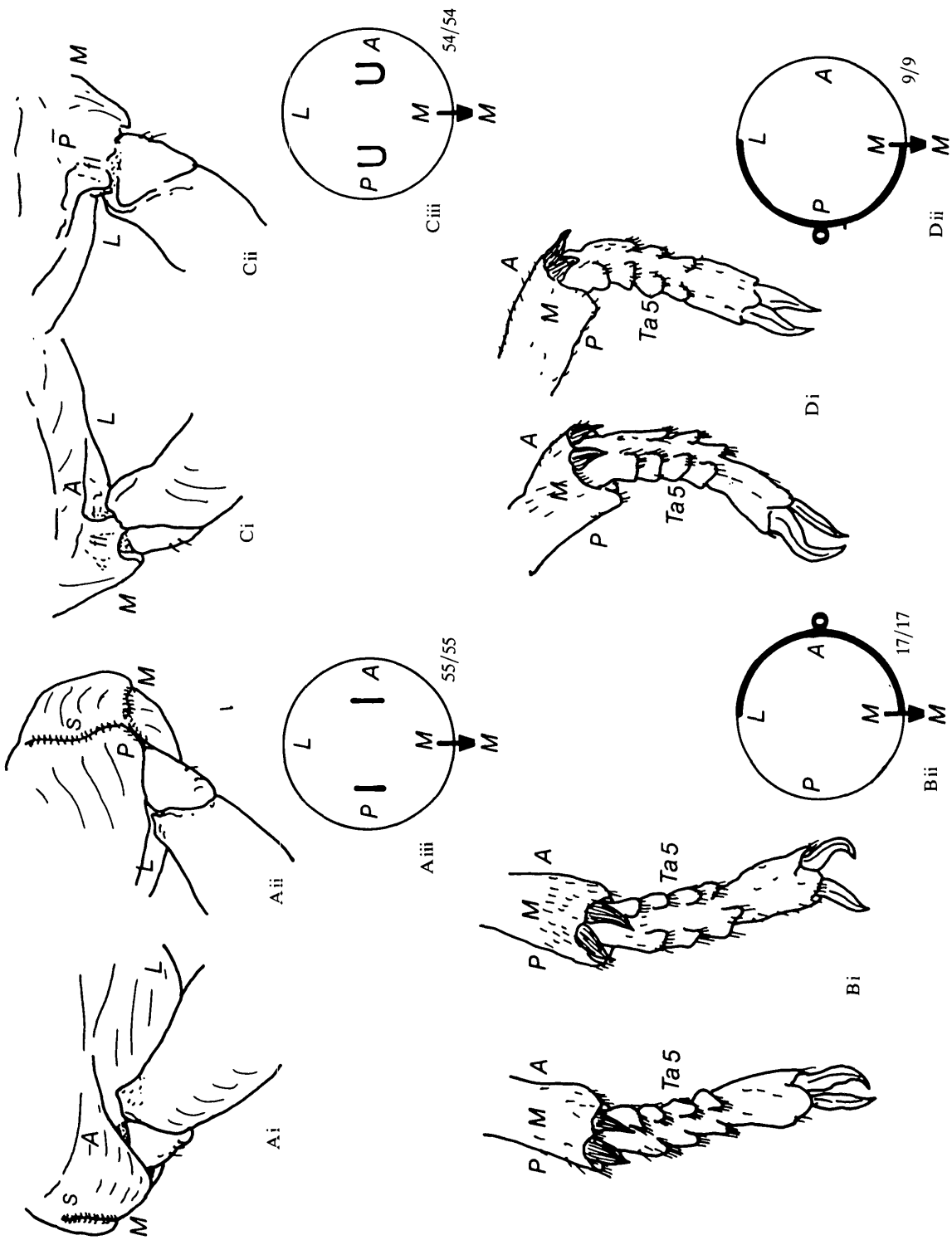
The *medial* supernumerary (Fig. 6A) could be analysed at the coxal apex in 55/59 cases and 22 of these appeared to resemble the host (metathoracic) coxa, having a flange on the anterior and a suture on the posterior face. In a further 13 cases the structure was consistent with a host-derived anterior (with flange) and a graft-derived posterior face (with flange), and in 2 cases the structure was consistent with graft-derived anterior and host-derived posterior (the coxa having sutures but no flanges on both faces). In the remaining 18 cases, flanges and/or sutures were partial or abnormal and the apex could not readily be interpreted. The tarsus could be analysed in 26 cases and was also of variable composition with the graft usually contributing tissue on the lateral side. The *lateral* supernumerary (Fig. 6B) exhibited a similar variability in structure at the level of the coxal apex, and the tarsus which usually had a graft contribution on the medial side (Fig. 6).

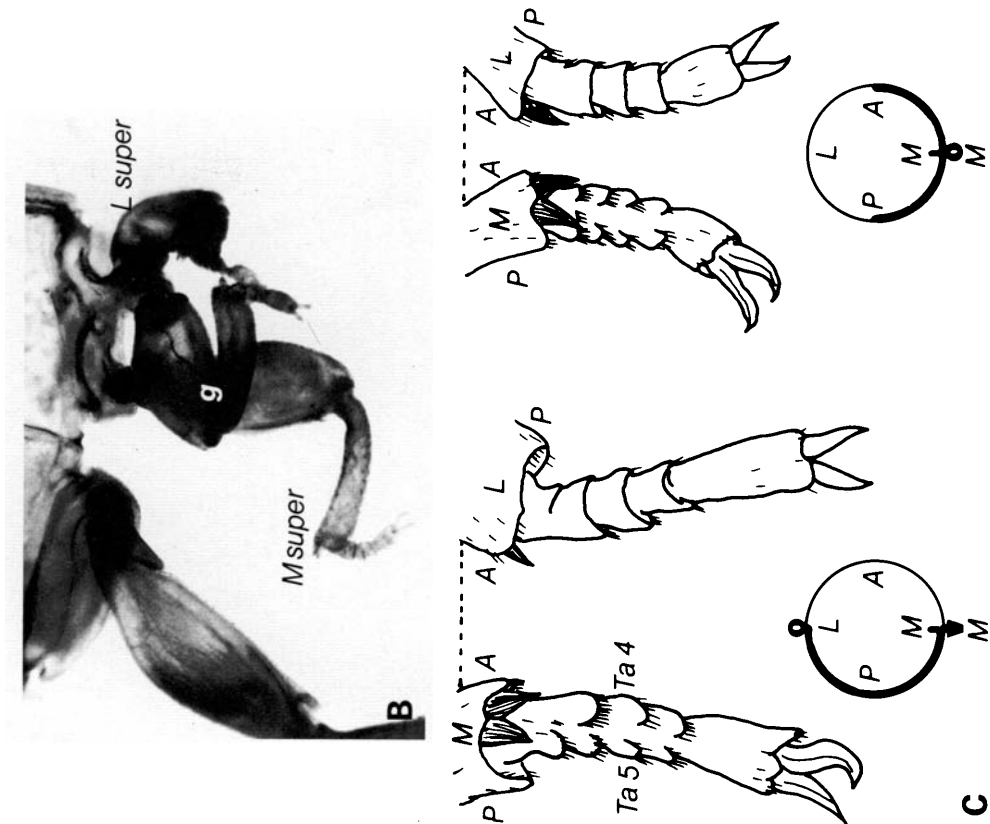
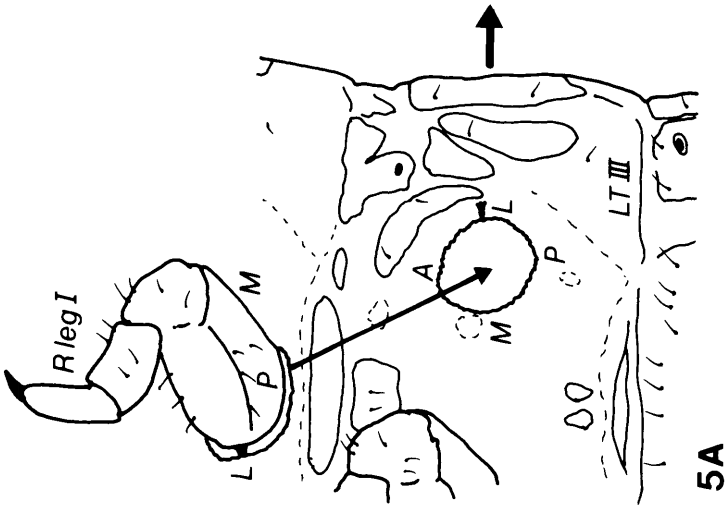
In the remaining 9/68 animals the grafted leg was not orientated with M/L axis reversed: the claws were directed posteriorly or anteriorly with respect to the host. In all cases two supernumeraries were formed (in lateral/posterior and medial/anterior, and in lateral/anterior and medial/posterior positions, respectively), they were orientated approximately like the host and were variable in composition.

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Fig. 4. Structure of posterior (A and B) and anterior (C and D) supernumerary legs formed after A/P axis reversal of the graft leg. (A) Coxa of posterior supernumerary leg, shown in *camera-lucida* drawings of anterior (i) and posterior (ii) views of one specimen and in schematic 'end-on' view (iii). The sutures (*s*), denoted by a bar in (iii), on anterior and posterior faces in all interpretable cases (numbers given in iii) are consistent with anterior being derived from graft and posterior from host. (B) Tarsus of posterior supernumerary, shown in medial views of two specimens (i) and in schematic 'end-on' view (ii). The five tarsal segments (*Ta5*), denoted by a thick line in (ii), present on the anterior side, indicate that the tarsus is anterior graft/posterior host in origin. (C) Coxa of anterior supernumerary. The flanges (*f*) present on anterior and posterior faces (denoted by a 'U' in iii) are consistent with anterior being derived from host and posterior from graft. (D) Tarsus of anterior supernumerary. The five tarsal segments are present on the posterior side, indicating an anterior host/posterior graft origin. In the schematic diagrams the orientation of the supernumerary (claws pointing medially, *M*, on the host) is shown by the small arrow, and on the diagrams of the tarsi the small circle indicates the part of the circumference which (at more proximal levels) is continuous with the grafted leg.







## (iv) 180° rotation

The left prothoracic leg was grafted with a 180° rotation into the left metathoracic leg site (Fig. 7A). 13 animals were fixed after two larval moults and, in all cases, the grafted leg retained its orientation (claw pointing laterally) and was usually accompanied by one, two or three bulges or supernumerary legs in various positions.

In 85/94 successfully grafted animals developing to adult, the grafted prothoracic leg was still 180° rotated and was associated with supernumerary legs which varied in number (one in 10/85, two in 68/85 and three in 7/85 cases), in position, in orientation and in composition.

In the 68 animals with *two supernumeraries*, the most frequent positions were anterior–medial and medial (18 cases) or anterior–medial and posterior–lateral (22 cases), but many other combinations also occurred (Fig. 7B). The supernumeraries were variable in structure and difficult to score at the coxal level, so only the tarsal structure is described (Fig. 8). Anterior–medial supernumeraries were most frequent (54 cases) and all were orientated with claws pointing medially. The tarsus was analysable in only 21 cases and these were occasionally entirely of host type but usually had a graft contribution on the graft side (Fig. 8). Posterior–lateral supernumeraries (24 cases) were usually orientated with claws pointing approximately laterally (15/24) or approximately medially (7/24). The tarsus was of graft structure on the graft side and host structure on the host side in 3/4 analysable cases. Medial supernumeraries (29 cases) were usually orientated with claws pointing approximately medially (23/29), and the 9 scorable tarsi all had host structure on the anterior half (7/9) or a small anterior portion of their surface. Details of the orientations and compositions of the other minor classes of supernumerary are given in Fig. 8.

In 5 of the 10 animals with only *one supernumerary* there was loss of most of the sclerites anterior to the graft, and a single posterior supernumerary usually orientated with claws pointing laterally. In the other 5 animals the sclerites were normal and the single supernumerary was in posterior–lateral (1), anterior–medial (1), anterior (1) or lateral (2) position, usually orientated with claws pointing medially.

7 animals had *three supernumeraries* (1 having two additional rudimentary outgrowths), which were usually in anterior–medial (7 cases) or medial (3 cases) positions, orientated with claws pointing medially, although several other positions and orientations were found (Fig. 7C). In some of these animals, (and in

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Fig. 5. Leg-to-leg site: medial–lateral axis reversal. (A) Schematic view of the operation, grafting the right prothoracic leg into the left metathoracic leg site, reversing the M/L axis. (B) Anterior view of resulting adult thorax showing the majority result with the grafted leg (g) plus medial (*M super*) and lateral (*L super*) supernumerary legs. (C) Tarsi of the medial and lateral supernumerary branches of one operated leg, shown in medial and lateral view and in schematic 'end-on' view (see Fig. 4). Each tarsus is approximately half-and-half in structure but they are *not* complementary (cf. Fig. 4).

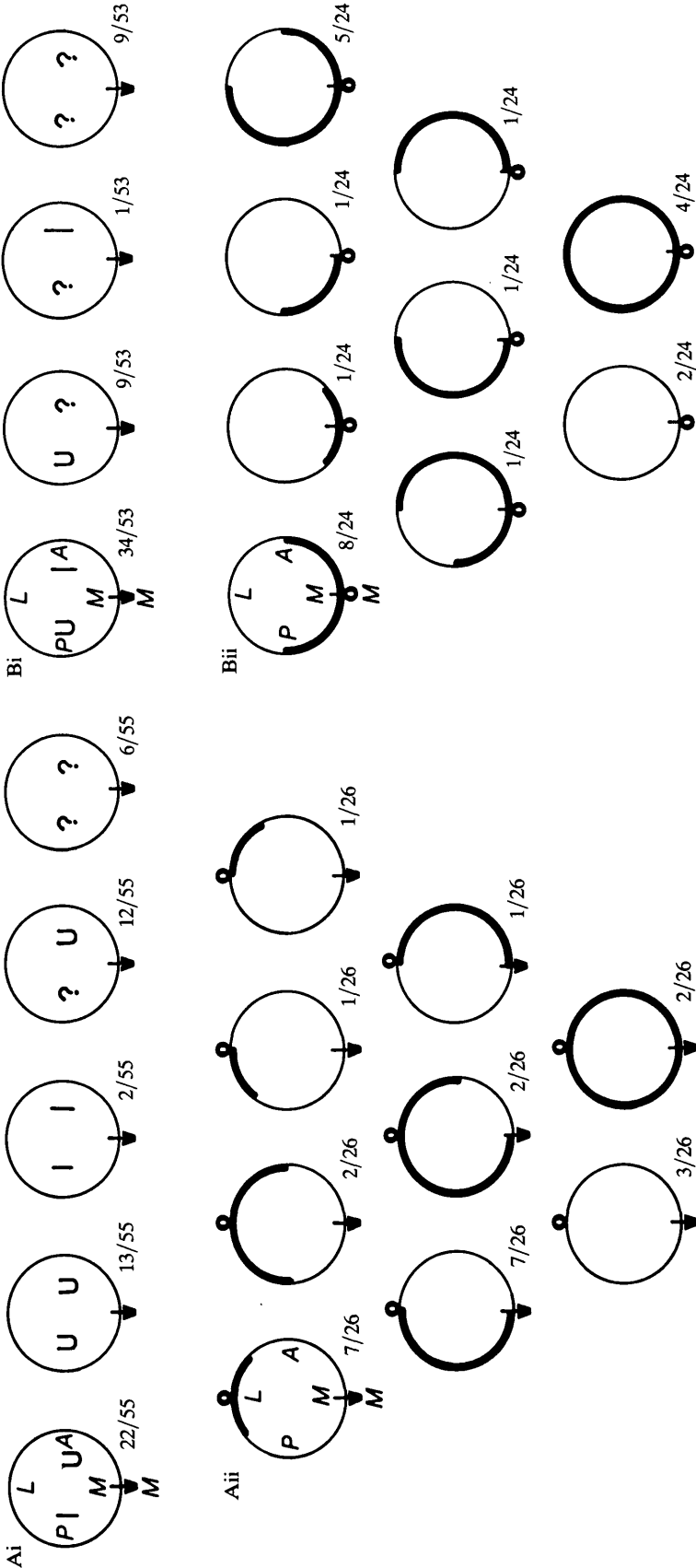


Fig. 6. Leg-to-leg site: medial-lateral axis reversal. Schematic 'end-on' views of (A) the medial supernumerary (scorable at (i) the coxal level in 55 cases, and (ii) the tarsal level in 26 cases) and (B) the lateral supernumerary (scorable at (i) the coxal level in 53 cases and at (ii) the tarsal level in 24 cases). Notations as in Figs 4, 5. All supernumeraries are orientated with claws pointing medially.

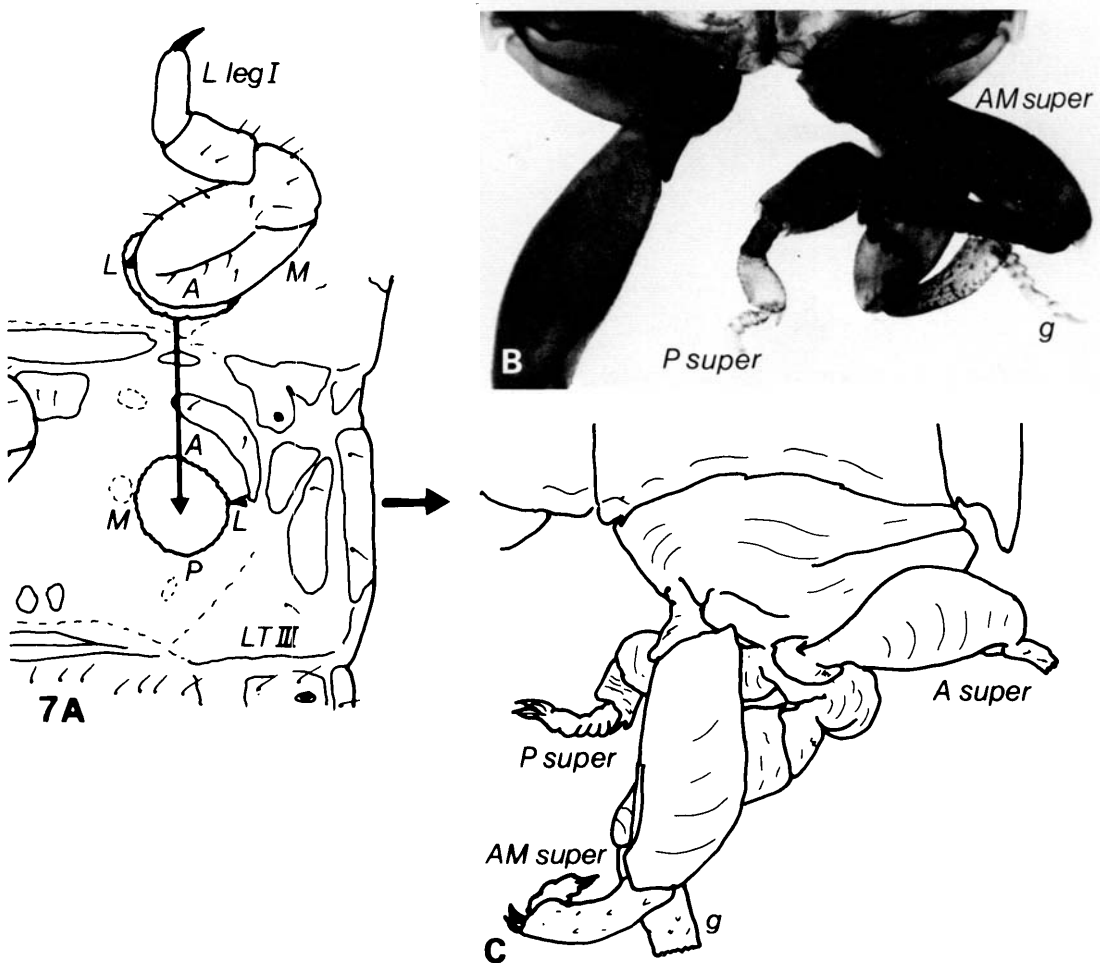


Fig. 7. Leg-to-leg site: 180° rotation. (A) Schematic view of the operation, grafting the left prothoracic leg rotated into the left metathoracic leg site. (B) Anterior view of resulting thorax of adult with two supernumeraries, showing the rotated graft (*g*), an anterior/medial supernumerary (*AM super*) orientated with claws pointing medially, and a posterior supernumerary (*P super*) orientated laterally. (C) Anterior view of adult with three supernumeraries, showing the broken rotated graft (*g*), an anterior/medial supernumerary (*AM super*) orientated medially, a posterior supernumerary (*P super*) orientated laterally, and a broken anterior supernumerary (*A super*) orientated medially.

some of those with two supernumeraries) two supernumeraries were fused at the base and only separated at the trochanter, femur or tibia level.

In the remaining 9/94 animals, the grafted prothoracic leg was orientated in alignment with host axes and accompanied, as in major class of results, by supernumerary legs varying in number (zero in 2/9), two in 6/9 and three in 1/9 cases), in position (most frequently anterior–medial or medial) in orientation and in composition.

The very variable results of the 180° rotation differ dramatically from those of the A/P axis reversal (where number, position, orientation and composition of supernumeraries are constant) and the M/L axis reversal (where only composition is variable). They also differ from most results of 180° rotation at coxal or tibial levels within the legs of other insects (see Introduction) where the graft *frequently* rotates back towards alignment and *frequently* forms no supernumeraries. To further investigate the effects of graft rotation in *Tenebrio*, the entire leg was rotated by only 90°.

(v) 90° rotation

The left prothoracic leg was grafted into the left metathoracic leg site with a 90° rotation so that the claw pointed anteriorly or posteriorly. 14 animals were fixed after moulting once and, in all cases, the grafted leg had rotated back towards alignment with the host axes and there were no signs of supernumerary structures.

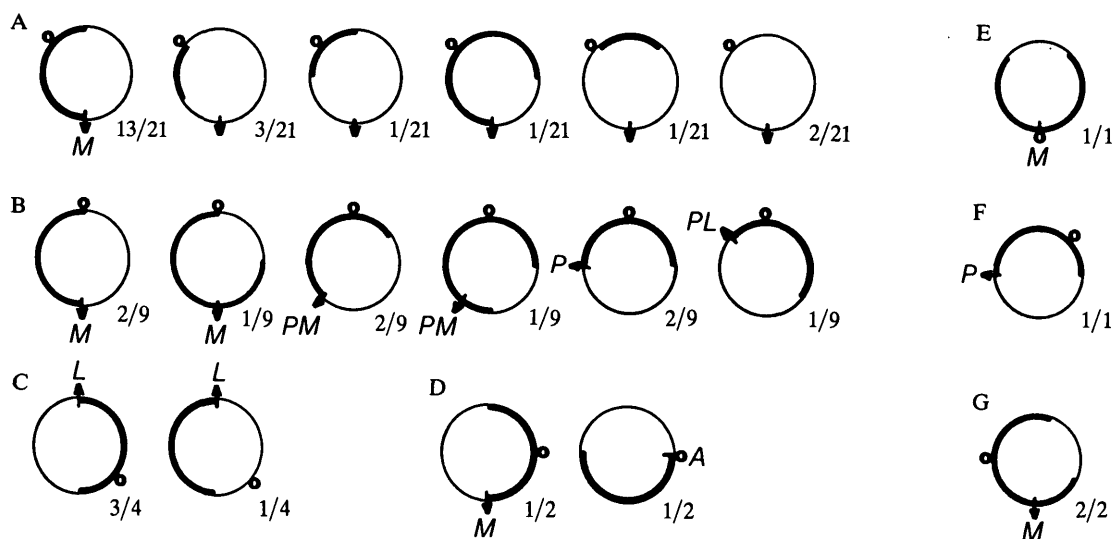


Fig. 8. Leg-to-leg site: 180° rotation. Details of the 68 animals forming two supernumeraries and having no derotation of the graft. The 136 supernumeraries are considered individually and shown in schematic 'end-on' view of the tarsus (as in Figs 4, 5, 6). (A) *Anterior/medial supernumeraries* (54 cases; orientated towards medial (M) in 53/54; unscorable 1/54; tarsi scorable in 21 cases). (B) *Medial supernumeraries* (29 cases; orientated M, 20/29; PM, 3/29; P, 3/29; PL, 1/29; unscorable 2/29; tarsi scorable in 9 cases). (C) *Posterior/lateral supernumeraries* (24 cases; orientated L, 13/24; M, 5/24; PL, 2/24; AM, 2/24; P, 1/24; unscorable, 1/24; tarsi scorable in 4 cases). (D) *Posterior supernumeraries* (14 cases; orientated L, 7/14; M, 2/14; P, 1/14; A, 1/14; AL, 1/14; unscorable, 2/14; tarsi scorable in 2 cases). (E) *Lateral supernumeraries* (6 cases; orientated M, 5/6; A, 1/6; tarsus scorable in 1 case). (F) *Posterior/medial supernumeraries* (5 cases; orientated L, 3/5; P, 2/5; tarsus scorable in 1 case). (G) *Anterior supernumerary* (4 cases; orientated M, 3/4; P, 1/4; tarsus scorable in 2 cases). Despite the variability in position and orientation of the supernumeraries, 38/40 scorable tarsi were of dual graft and host origin, usually with graft-derived tissue on the graft side of the supernumerary and often with the borders between graft- and host-derived tissue in midmedial and midlateral position.

In all 81 successfully grafted animals which developed to adult, the grafted prothoracic leg was aligned with host axes and in 73/81 cases there was no supernumerary structure. In 8 cases there was a supernumerary leg in anterior, posterior, anterior–medial or medial position. Supernumerary structures were orientated like the host and were usually broken or rudimentary.

Clearly, after a 90° rotation the leg can readily derotate and rarely forms supernumerary structures. The lack of rotation following the more extreme 180° rotation may be a curious feature of *Tenebrio* or a result of making the graft at the leg base and thereby studying interaction with the surrounding thorax. This was investigated by repeating the control, A/P reversal and 180° rotation grafts at the level of the leg femur.

### (C) Grafts at femur level

The left (series i, iii) or right (series ii) metathoracic leg was amputated in the proximal third of the femur and the distal part was grafted to the amputated mid-femur of the left prothoracic leg of the same animal. The slight size difference between the legs enabled the graft to be telescoped inside the stump.

#### (i) Control orientation

The left metathoracic femur was grafted without rotation into the left prothoracic femur. In all 31 animals which retained the graft and developed to adult, the distal part of the leg was normally orientated and of graft (metathoracic) structure with tibial spines directed anteriorly and a four-segmented tarsus (29/31 cases: the others were poorly segmented). There were no supernumerary structures.

#### (ii) Anterior–posterior reversal

The right metathoracic femur was grafted with A/P reversal to the left prothoracic femur (Fig. 9A). 39 animals developed to adult with undamaged interpretable legs and in 38/39 cases the graft retained its orientation and was accompanied by supernumerary structures. The supernumeraries usually (22/38 cases) branched from the main leg at distal femur level (as in Fig. 9B,C) but sometimes they separated in the tibia (Fig. 9D) or after graft and host had independently regenerated to tibia level).

In 16/38 cases, separate supernumeraries were formed in approximately anterior and posterior positions and were all orientated like the host, claws directed medially. As in the case of A/P supernumeraries generated at the base of the leg, these femur-level supernumeraries were very *consistent* in composition. The tarsus of the anterior supernumerary had five segments (like the host) on the anterior side and four (like the graft leg) on the posterior side in all 13 analysable cases. The posterior tarsus was composed of four segments on the anterior side and five on the posterior side (14/15 analysable cases – one tarsus was largely four-segmented with only a small five-segmented region on the medial side).

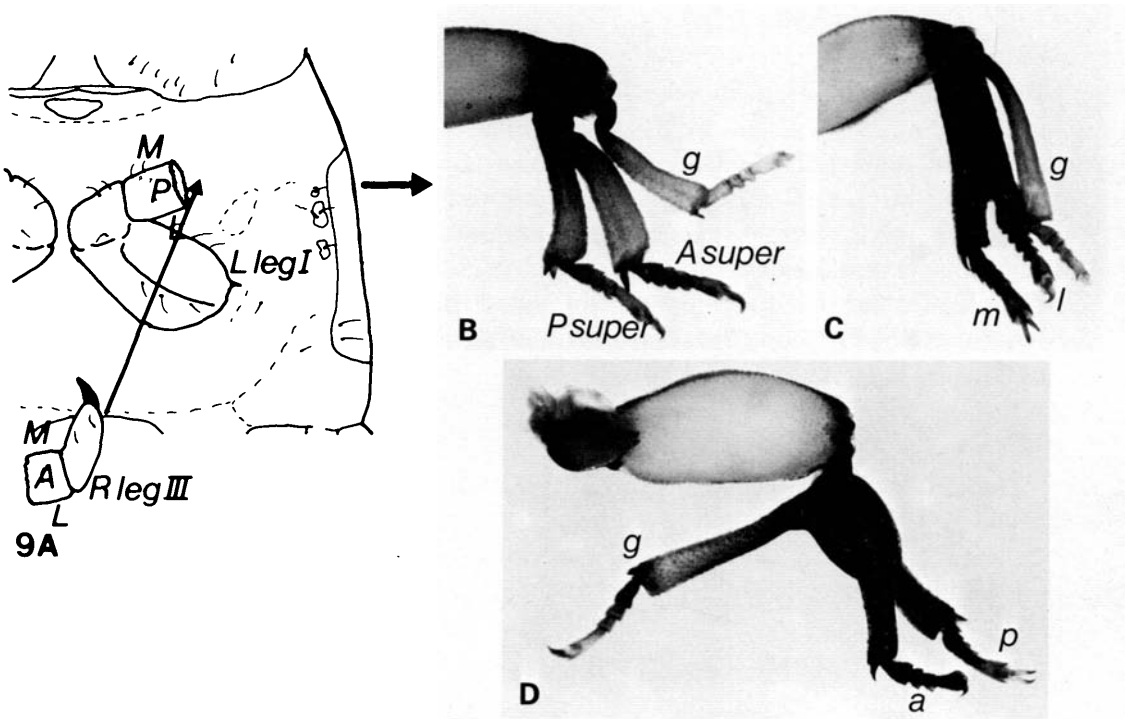


Fig. 9. Femur level: anterior-posterior axis reversal. (A) Schematic view of the operation, grafting the distal part of the right metathoracic leg (*R leg III*) into the femur of the left prothoracic leg (*L leg I*), reversing the A/P axis. (B) Anterior view of resulting adult leg with separate anterior (*A super*) and posterior (*P super*) supernumeraries, each orientated with claws pointing medially and half-graft/half-host in structure. (C) Anterior view of adult leg with a fused double supernumerary in a medial position at distal femur level. The supernumerary separates distally into a medial tarsus (*m*) orientated more-or-less posteriorly, and a lateral tarsus (*l*) orientated more-or-less anteriorly. Both tarsi are half-and-half in structure. (D) Anterior view of adult leg with a fused double supernumerary in a lateral position at proximal tibia level. The supernumerary separates into an anterior tarsus (*a*) orientated more-or-less anteriorly and a posterior tarsus (*p*) orientated more-or-less posteriorly. Both tarsi are half-and-half in structure.

In the other 22/38 cases the two supernumeraries were fused into a large double structure in medial (15 cases), lateral (6 cases) or anterior-medial (1 case) position. In most cases the supernumeraries branched more distally into tarsi which were half graft/half host and strictly complementary in structure. Further details of these fused supernumeraries are given in Fig. 10.

In the remaining animal the graft was misaligned, with claws directed more-or-less laterally, and bore a fused supernumerary in anterior position, which separated into medial and lateral tarsi, each half host/half graft in structure.

### (iii) 180° rotation

The left metathoracic femur was grafted 180° rotated to the left prothoracic femur (Fig. 11A). In all 49 successfully grafted animals which developed to adult,



the graft had derotated more-or-less into alignment with the host leg. In 24/49 cases there were *no* supernumeraries (Fig. 11B) and in 24/49 cases there were interpretable supernumerary structures (the remaining animal had extensive fusion between graft and supernumerary).

The supernumeraries were very variable in structure, position, orientation and composition. In 9/24 cases there was a tapering, *distally incomplete* structure, ranging from a rudimentary femur apex to femur apex plus a thin tibial (Fig. 11C) and (in one case) tarsal lobe, and positioned laterally or anteriorly on the graft/host junction. In 1/24 cases there was a *single complete* supernumerary, in anterior position and with host orientation and tarsal structure. In 7/24 cases there

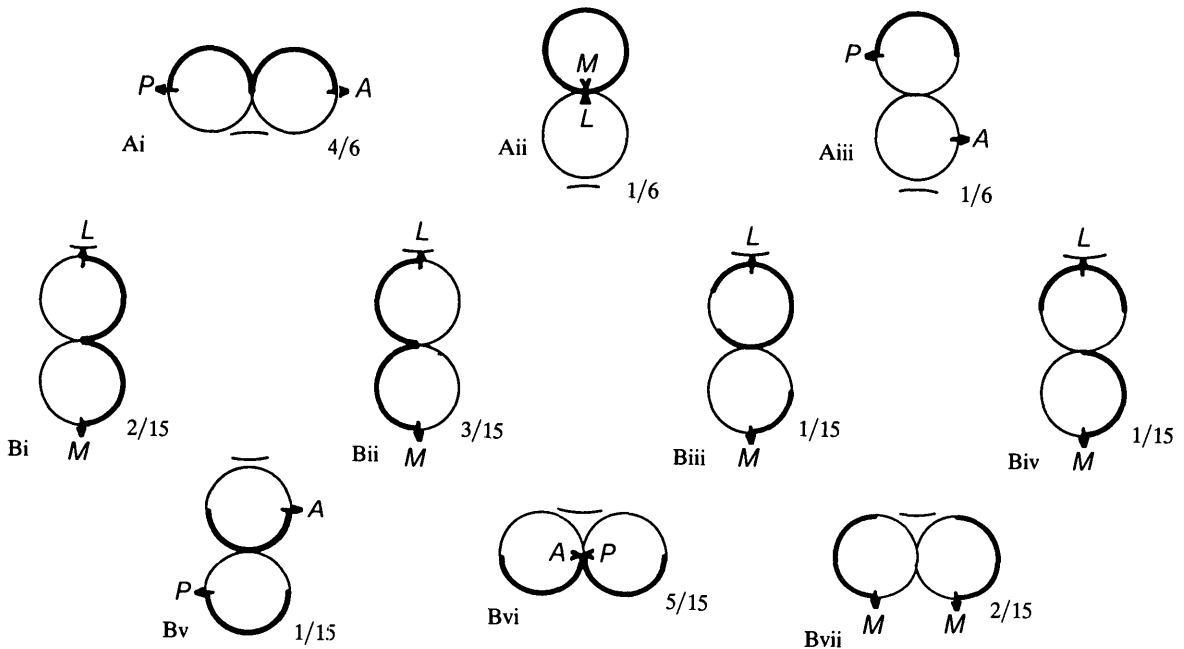


Fig. 10. Femur level: A/P axis reversal. Details of the 22 animals forming a fused double supernumerary and having no misalignment of the graft. The supernumerary tarsi are shown in schematic 'end-on' view (as in Fig. 4) with parts which are five-segmented (host-type in these femur-level experiments) shown by thick lines. The small arc indicates the part of the circumference which (at more proximal levels) is continuous with the graft. (A) *Laterally fused supernumeraries* (6 cases) branch into (i) posterior tarsus orientated posteriorly and anterior tarsus orientated anteriorly, (ii) lateral tarsus orientated medially and medial tarsus orientated laterally or (iii) lateral tarsus orientated posteriorly and medial tarsus orientated anteriorly. (B) *Medially fused supernumeraries* (15 cases) branch into (i–iv) lateral tarsus orientated laterally and medial tarsus orientated medially, (v) lateral tarsus orientated anteriorly and medial tarsus orientated posteriorly, (vi) posterior tarsus orientated anteriorly and anterior tarsus orientated posteriorly, or (vii) anterior and posterior tarsi both orientated medially. There was also one non-scorable anterior/medially fused double supernumerary. In 19/21 cases each component tarsus was of dual graft- and host-origin, in 18/21 cases the structures of the two component tarsi were strictly complementary and in 17/21 cases the graft/host borders on each tarsus were in midmedial and midlateral positions.

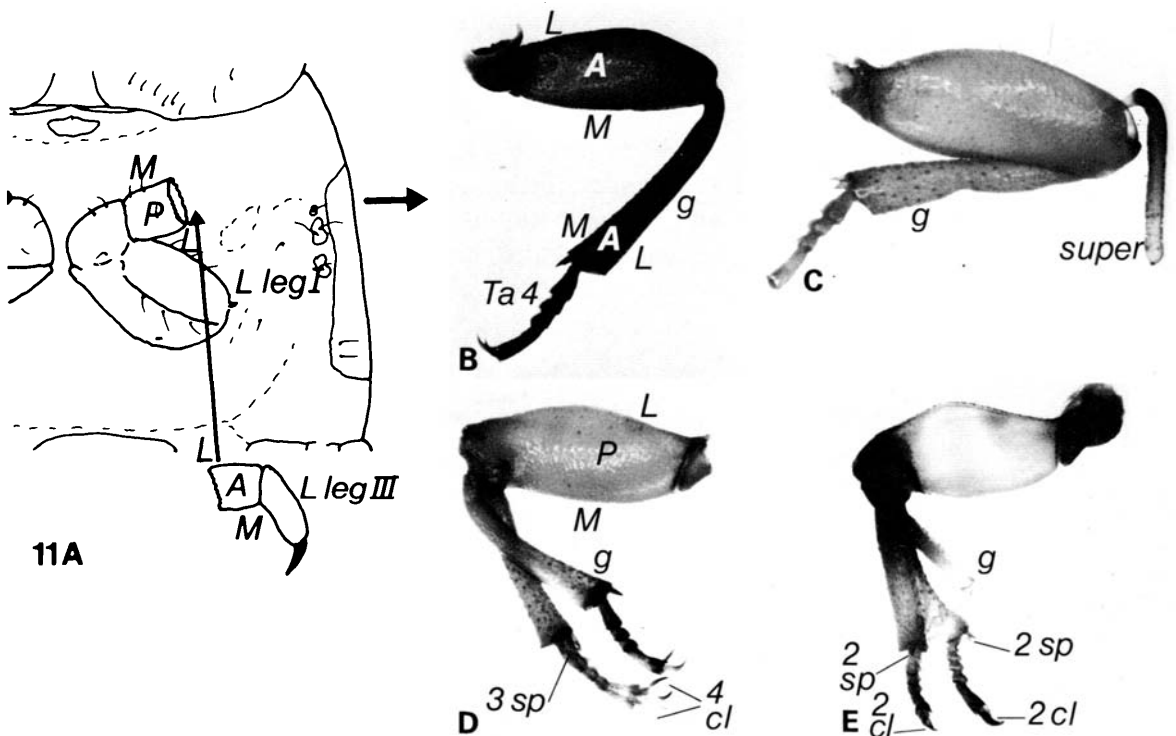


Fig. 11. Femur level: 180° rotation. (A) Schematic view of the operation, grafting the distal part of the left metathoracic leg, 180° rotated, into the femur of the left prothoracic leg. (B) Anterior view of resulting adult leg with derotated graft (*g*) of metathoracic tarsal structure (*Ta 4*), and no supernumerary. (C) Anterior view of leg with a supernumerary partial femur apex and tibial lobe (*super*). (D) Posterior view of leg with a diverging supernumerary with three spines on the distal tibia (*3sp*) and terminating in two complete sets of claws (*4cl*). (E) Posterior view of leg with a double supernumerary separating into two branches each with two tibial spines (*2sp*) and a set of claws (*2cl*).

was a *diverging double* supernumerary which had no spines, one spine or three spines at distal tibia level, but bore a fused tarsus with three claws or two separate complete tarsi in various positions and orientations (Fig. 11D). The remaining 7/24 animals bore a complete *double fused* supernumerary with four distal tibial spines and two separate tarsi which, again, were variable in position, orientation and composition (Fig. 11E).

#### DISCUSSION

In the major series of experiments the *Tenebrio* larval leg (plus a narrow ring of surrounding thorax) was removed and grafted into the leg site, with the transverse axes aligned or misaligned with those of the host body segment. The results show that interactions occurring at the base of the leg can provoke the regeneration of

supernumerary larval and eventually adult leg structures similar to those resulting from comparable grafts performed at femur level within the *Tenebrio* leg and at distal coxa or tibia level within the legs of a variety of hemimetabolous insects.

The development of legs in holometabola such as *Tenebrio* and Lepidoptera differs from that in hemimetabola in that the size, proportions, cuticular patterns and segmentation of larval and adult legs are very different (e.g. Fig. 1). The origin of the lepidopteran adult leg is not completely resolved (Bodenstein, 1937, 1941; Kuske, 1963) and it may be derived from only part of the larval leg. In *Tenebrio*, however, Huet & Lenoir-Rousseaux (1976) showed clearly that the epidermis of the whole larval leg forms that of the adult leg. There is considerable correspondence between larval and adult segments, except that the trochanter also forms the proximal part of the adult femur, and the tibia also forms most of the adult tarsus. This correlates well with the level of supernumerary adult structures after grafts on larval legs: interactions at the base of the larval leg produce supernumeraries at the base or within the adult coxa, while interactions in the larval femur give supernumeraries from the adult distal femur or proximal tibia.

Previous grafting operations on the larval legs of *Tenebrio* (Balazuc, 1948) and various Lepidoptera (Bodenstein, 1937, 1941) have involved the contralateral exchange or 180° rotation of the distal part of the leg, and have produced supernumerary leg structures. It is difficult, however, to compare directly the results with those from hemimetabolous insects since the position and orientation of supernumeraries was not recorded and related to the precise grafting operation. Here I show that the femur-level control, A/P-reversed and 180°-rotated *Tenebrio* grafts do give similar results to those from cockroaches, crickets and stick insects. Control grafts simply heal, while A/P reversed grafts maintain their orientation and form either separate anterior and posterior supernumeraries or a fused double supernumerary in medial or lateral position. The range of positions and orientations of the component tarsi of the double supernumeraries (Fig. 10) accord well with those of comparable cockroach (French, 1976) and cricket (French, 1984) legs, and support the contention (French, 1976) that the double supernumeraries result from a fusion of separate supernumeraries arising in anterior and posterior positions. 180°-rotated *Tenebrio* femur grafts derotate and sometimes form supernumeraries in various positions and with structures ranging from a symmetrical partial femur apex to a complete fused double supernumerary.

In his experiments on *Tenebrio* legs, Balazuc (1948) described the frequent formation of outgrowths from the femur or tibia of *metathoracic* legs after grafts performed on the *mesothoracic* legs! This bizarre 'effet à distance' was never observed in this study and probably resulted from incidental damage, perhaps caused by forceps used in holding the animals.

When the A/P axis of the entire leg is reversed with respect to the surrounding thorax, interaction between the posterior edge of the leg base and thoracic epidermis anterior to the leg site results in regeneration of a supernumerary leg. Similarly, a second supernumerary forms between the anterior edge of the leg base and the posterior thorax. In a related experiment, the extirpation of a transverse

strip including the larval leg of *Tenebrio* or the cockroach (Bohn, 1974) allows the interaction of anterior and posterior thoracic epidermis and results in regeneration of the leg. Furthermore, when a transverse band of thorax is removed between successive cockroach (Bohn, 1974) or *Tenebrio* (French & Rowlands, unpublished) legs, a supernumerary leg is formed in reversed A/P orientation. All of these experiments show that pattern regulation occurs in the A/P axis of the thoracic segment, just as interaction between anterior and posterior epidermis of the abdominal segment results in regeneration of structures normally lying in the centre of the segment (Stumpf, 1966; Lawrence, 1973).

When the M/L axis of the *Tenebrio* leg is reversed, supernumerary legs are formed at the confrontation of medial leg base and lateral thorax, and lateral leg base and medial thorax. Pattern regulation has not previously been demonstrated in the M/L axis of the insect ventral thorax and, in general, very little is known about the M/L axis of the insect segment since, unlike the A/P axis, it does not usually have useful markers of polarity or position. Shelton (1979) has studied the cockroach ecdysial line, which is in the dorsal midline of the thoracic segments. This structure can be regenerated after an excision or grafting experiment which confronts, with opposite mediolateral polarities, regions from close to the midline. However, the ecdysial line lies at a boundary of a M/L axis and it may form by mechanisms not typical of pattern regulation in the rest of the axis. The regeneration of supernumerary crustacean legs has been shown by grafts which disrupt the M/L axis. When the basipodite (second segment) of the crayfish leg is grafted to a site on the ventral midline between the legs, an extra leg is regenerated at the confrontation of medial thorax and the lateral face of the leg (Mittenthal & Trevarrow, 1984).

The position and orientation of entire A/P and M/L supernumerary *Tenebrio* legs is similar to that of supernumeraries arising at a distal level on the leg, and their composition also seems similar. The composition of supernumeraries has been investigated by using differences in cuticular colour between species (Bohn, 1972; French, in preparation) or differences in cuticular pattern between prothoracic and metathoracic legs (Bulliere, 1970; French, 1984). The differences between the *Tenebrio* legs are reliably found in regenerated structures, but they are limited to certain areas and can only give an indication of the composition of the supernumeraries. The proximal difference lies in the locations of the suture and flange, which are complex features of the anterior and posterior sides of the coxa/trochanter joint. The marker is useful in distinguishing the graft from supernumerary legs and in analysing the A/P supernumeraries (Fig. 4) where the anterior and posterior sides are of different origins. However it is difficult to use coxal structure in analysing the M/L and 180° supernumeraries when the anterior and posterior sides are often of dual origin and abnormal or partial sutures and flanges are formed. The distal marker consists of the subdivision of the proximal part of the prothoracic tarsus into two segments, giving five tarsal segments in all. This can be analysed around most of the tarsal circumference but it only enables

some legs to be scored, as supernumeraries often have their distal parts poorly developed or broken off.

When analysed at coxa and tarsus level, the A/P supernumeraries are found to be *consistent* and *complementary* in structure. The anterior leg is of host (meta-thoracic) structure on the host (anterior) side and of graft structure on the posterior side, while the posterior leg is of graft (prothoracic) structure on the graft (anterior) side and of host structure on the posterior side. The borders between host- and graft-derived parts seem to fall in constant positions on the tarsus, approximately midmedial and midlateral, as in the A/P supernumeraries formed at femur level in the *Tenebrio* leg and at tibia level in the cricket leg (French, 1984; French & Toussaint, in preparation). This result, together with the restriction found in circumferential intercalary regeneration in the cockroach leg (French, 1980), suggests that the insect larval leg, like the *Drosophila* leg disc (Steiner, 1976), is divided into A and P compartments, and that these are respected during regeneration. The fact that this consistent composition is seen in legs formed between thorax and leg base suggests that the division into A and P compartments may extend from the leg across the thoracic segment.

After M/L reversal of the grafted leg, the supernumerary legs are *variable* and often *non-complementary* in structure (Figs 5, 6), as was also found in M/L supernumeraries formed at tibia level in the cricket leg (French, 1984). The *Tenebrio* M/L supernumeraries did *not* show the restricted range of structures found in the cricket supernumeraries, where medial legs were often of pure graft (but never host) or of A graft/P host (but never the reverse) structure, and lateral ones were often of pure host or A host/P graft structures. The variable structures of M/L supernumeraries and the circumferential intercalation results from the cockroach leg (French, 1980) give *no* indication that the insect leg or thoracic segment is subdivided into medial and lateral compartments.

After 180° rotation of the entire *Tenebrio* leg the results differ somewhat from those found at various levels within the legs of *Tenebrio* and other insects. The grafted leg *rarely* (9/94) derotates back into alignment with the host, although derotation readily occurs if the grafted leg is only turned through 90°. Supernumerary legs are *always* formed (except in 2 cases where the graft derotated), they are two (or occasionally one or three) in number, in a wide variety of positions and orientations (Fig. 8), and almost always of dual graft- and host-origin. The formation of supernumeraries at the different positions seems largely independent since a variety of different combinations occurs, and the relative frequencies of the different positions are approximately equal in the one-, two- and three-supernumerary classes of animal. 180° rotation reverses both A/P and M/L axes of the grafted leg, but the positions and orientations of supernumeraries clearly depend on the overall pattern of interaction between the rotated leg base and its surrounds, rather than being a sum of the independent effects of reversing an A/P and an M/L axis (see Fig. 12).

The details of the positions and orientations of the supernumeraries formed after the 180° rotation are not easily reconciled with either of the two models

currently proposed for the formation of insect supernumerary legs. In the *polar co-ordinate model* supernumeraries form from complete circumferences generated by intercalary regeneration between graft and host (French *et al.* 1976). If circumferential values are spaced evenly on leg and thorax, a  $180^\circ$  leg rotation will give zero, two or four or more supernumeraries, in any positions. Those forming medially or laterally will be orientated towards medial, while those forming anteriorly and posteriorly will be orientated either towards medial or lateral. Uneven distribution of values will give two supernumeraries, with constant opposite locations and constant orientations (French *et al.* 1976). In the *boundary model* (Meinhardt, 1983, 1984) the thorax and leg are divided into A and P and into M and L compartments and a leg is initiated where the boundaries cross, i.e. wherever three (or possibly four) compartments come into contact. After  $180^\circ$  rotation the three-compartment model (Meinhardt, 1983) predicts supernumeraries of normal structure in anterior and posterior positions (orientated towards medial and lateral, respectively) plus double supernumeraries in lateral

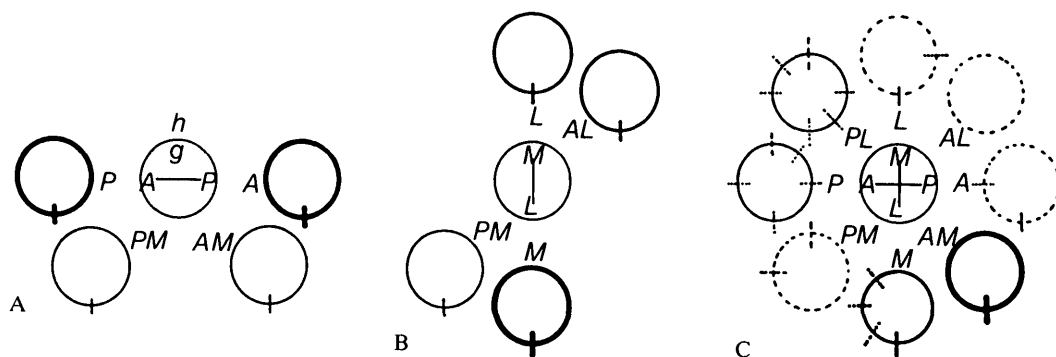


Fig. 12. The positions and orientations of supernumeraries formed after (A) A/P reversal, (B) M/L reversal and (C)  $180^\circ$  rotation. The inner circle represents the grafted leg (g), showing the reversed axes, and the outer circles on the surrounding thorax (h) represent the supernumeraries. Heavy circles are positions occupied by  $>33\%$  of the supernumeraries, medium circles =  $20-30\%$ , light circles =  $10-20\%$  and light-dashed circles are the positions in which less than  $10\%$  of supernumeraries are found. On the circles, lines indicate orientation; solid lines are orientation of  $>75\%$ , dashed lines of  $25-75\%$  and dotted lines of less than  $25\%$  of supernumeraries formed at that site. (A) A/P axis reversal – 61 animals with graft orientated medially and no extensive damage; 122 supernumeraries. (B) M/L axis reversal – 59 animals with graft orientated laterally; 118 supernumeraries. (C)  $180^\circ$  rotated – 80 animals with graft orientated laterally and no extensive damage; 162 supernumeraries. The  $180^\circ$  rotation is not equivalent to reversing independent A/P and M/L axes since (i) some major sites of supernumeraries after reversal of a single axis (L, AL, A) are very minor sites after the  $180^\circ$  rotation, (ii) a fairly common site after  $180^\circ$  rotation (PL) is never occupied after reversal of a single axis, (iii) many sites which are occupied after one of the axis reversals or the  $180^\circ$  rotation (P, M, PM) have a constant orientation after the axis reversal but a variable orientation after  $180^\circ$  rotation (and in the case of the P and PM sites the most frequent orientation differs from that found after single axis reversal) (iv) the composition of A and P supernumeraries is not the same after  $180^\circ$  rotation as after A/P axis reversal.

and medial positions (orientated towards posterior/lateral and anterior/medial, respectively). A four-compartment model generates predictions similar to those of the polar co-ordinate model with even spacing.

## CONCLUSION

The similarity in the position, orientation and composition of supernumeraries found after reversing one transverse axis at the base of the beetle leg and at a more distal level within the leg, suggest a similar organization of positional values and compartments in the epidermis of the leg and the surrounding thoracic body segment. The details of the results of the 180° rotation, however, cannot be satisfactorily explained at present.

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## REFERENCES

- BALAZUC, J. (1948). La tératologie des coléoptères et expériences de transplantation sur *Tenebrio molitor* L. *Mém. Mus. natn. Hist. nat. Paris* (Ser. A) **25**, 1–193.
- BART, A. (1971). Morphogenèse surnuméraire au niveau de la patte du Phasme *Carausius morosus* Br. *Wilhelm Roux Arch. EntwMech. Org.* **166**, 331–364.
- BODENSTEIN, D. (1937). Beintransplantationen an Lepidopterenraupen. IV. Zur Analyse experimentell erzeugter Bein-Mehrfachbildungen. *Wilhelm Roux Arch. EntwMech. Org.* **136**, 745–785.
- BODENSTEIN, D. (1941). Investigations on the problem of metamorphosis. VIII. Studies on leg determination in insects. *J. exp. Zool.* **87**, 31–53.
- BOHN, H. (1965). Analyse der Regenerationsfähigkeit der Insekten-extremität durch Amputations- und Transplantationsversuche an Larven der afrikanischen Schabe *Leucophaea maderae* Fabr. (Blattaria). II. Mitt. Achsensdetermination. *Wilhelm Roux Arch. EntwMech. Org.* **156**, 449–503.
- BOHN, H. (1970). Interkalare Regeneration und segmentale Gradienten bei den Extremitäten von *Leucophaea* – Larven (Blattaria). I. Femur und Tibia. *Wilhelm Roux Arch. EntwMech. Org.* **165**, 303–341.
- BOHN, H. (1972). The origin of the epidermis in the supernumerary regenerates of triple legs in cockroaches (Blattaria). *J. Embryol. exp. Morph.* **28**, 185–208.
- BOHN, H. (1974). Extent and properties of the regeneration field in the larval legs of cockroaches (*Leucophaea maderae*). I. Extirpation experiments. *J. Embryol. exp. Morph.* **31**, 557–572.
- BULLIÈRE, D. (1970). Interprétation des régénérats multiples chez les Insectes. *J. Embryol. exp. Morph.* **23**, 337–357.
- FRENCH, V. (1976). Leg regeneration in the cockroach, *Blattella germanica*. II. Regeneration from a non-congruent tibial graft/host junction. *J. Embryol. exp. Morph.* **35**, 267–301.
- FRENCH, V. (1978). Intercalary regeneration around the circumference of the cockroach leg. *J. Embryol. exp. Morph.* **47**, 53–84.
- FRENCH, V. (1980). Positional information around the segments of the cockroach leg. *J. Embryol. exp. Morph.* **59**, 281–313.
- FRENCH, V. (1984). The structure of supernumerary leg regenerates in the cricket. *J. Embryol. exp. Morph.* **81**, 185–209.
- FRENCH, V., BRYANT, P. & BRYANT, S. (1976). Pattern regulation in epimorphic fields. *Science* **193**, 969–981.
- HUET, C. & LENOIR-ROUSSEAU (1976). Etude de la mise en place de la patte imaginaire de *Tenebrio molitor*. I. Analyse expérimentale de restauration au cours de la morphogenèse. *J. Embryol. exp. Morph.* **35**, 303–321.

- KUSKE, G. (1963). Untersuchungen zur Metamorphose der Schmetterlingsbeine. *Wilhelm Roux Arch. EntwMech. Org.* **154**, 354–377.
- LAWRENCE, P. (1973). The development of spatial patterns in the integument of insects. In *Developmental Systems: Insects*, vol. 2 (ed. S. J. Counce & C. H. Waddington), pp. 157–209. London, New York: Academic Press.
- LENDER, T. & GROBOCOPATELI, A. (1967). Etude du territoire de régénération de la patte larvaire de *Tenebrio molitor* (Coleoptere). *Bull. Soc. Zool. Fr.* **92**, 213–222.
- MEINHARDT, H. (1983). Cell determination boundaries as organizing regions for secondary embryonic fields. *Devl Biol.* **96**, 375–385.
- MEINHARDT, H. (1984). Models for positional signalling, the threefold subdivision of segments and the pigmentation pattern of molluscs. *J. Embryol. exp. Morph.* **83 Suppl**, 289–311.
- MITTENTHAL, J. & TREVARROW, W. (1984). Intercalary regeneration in legs of crayfish: proximal segments. *Devl Biol.* **101**, 40–50.
- NÜBLER-JUNG, K. (1977). Pattern stability in the insect segment. I. Pattern reconstitution by intercalary regeneration and cell sorting in *Dysdercus intermedius* Dist. *Wilhelm Roux' Arch devl Biol.* **183**, 17–40.
- SHELTON, P. (1979). Post-embryonic determination of the ecdysial line in the cockroach: evidence for pattern regulation in the medio-lateral axis. *J. Embryol. exp. Morph.* **49**, 27–46.
- STEINER, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *Wilhelm Roux' Arch. devl Biol.* **180**, 9–30.
- STUMPF, H. (1966). Mechanisms by which cells estimate their location within the body. *Nature, Lond.* **212**, 430–431.
- WOLPERT, L. (1971). Positional information and pattern formation. *Curr. Top. Devl Biol.* **6**, 183–224.
- WRIGHT, D. & LAWRENCE, P. (1981). Regeneration of the segment boundary in *Oncopeltus*. *Devl Biol.* **85**, 317–327.

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