The structure of supernumerary leg regenerates in the cricket

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SUMMARY

In many insects, grafting a larval leg onto the contralateral leg stump reverses one transverse axis and leads to the regeneration of supernumerary legs at each of the two points of maximum discrepancy on the graft/host junction. These operations were performed on the cricket *Acheta domesticus*, grafting between tibiae of pro- and metathoracic legs, and between tibia and tarsus, in order to deduce the mode of origin of the supernumeraries from their graft type, host type or composite structure.

Supernumerary legs formed after A/P axis reversal are *always half-and-half* in structure, being of host type on the host side and graft type on the graft side, while supernumeraries formed after M/L axis reversal are *variable* in structure. These results are generally consistent with the recent Polar Co-ordinate Model (whereby supernumeraries form because the pattern of intercalation between graft and host generates two complete circumferences at the junction), provided that intercalation is restricted by the borders between anterior and posterior leg compartments. However there are features of the structure of the M/L supernumeraries which the Polar Co-ordinate Model does *not* explain. Medial supernumeraries are often of pure graft type (and lateral ones of host type) or half-and-half with anterior graft type and posterior of host type (while lateral supernumeraries often have the converse structure).

INTRODUCTION

Insect legs bearing supernumerary branches are occasionally found in nature (see Bateson, 1894) and were first generated experimentally by Bodenstein (1937), who amputated the legs of moth larvae, rotated them and replaced them on their stumps. Since then, legs with supernumerary branches have been produced by similar grafting experiments on the larvae of other holometabolous insects such as beetles (Balazuc, 1948; French, in prep.), and, especially, of hemimetabola such as cockroaches (Bohn, 1965, 1972; Bullière, 1970; French, 1976), stick insects (Bart, 1971) and bugs (Shaw & Bryant, 1975). Supernumerary structures are most reliably produced by grafting the distal parts of a leg onto the contralateral stump, so as to reverse the anterior–posterior (A/P) or medial–lateral (M/L) axis of the graft relative to the host. A set of distal leg parts with host orientation is typically regenerated from the graft/host junction at each site of axis discontinuity (e.g. in anterior and posterior positions after an A/P axis reversal).

In order to interpret the formation of these regenerates it is important to know not only their position and orientation, but also whether they are derived from tissue of the graft, the host or both. In attempts to investigate this, grafts have been performed between pro- and metathoracic legs of slightly different structure (Bullière, 1970; Bart, 1971), or between the legs of differently-coloured mutants (French, 1976) or species (Bohn, 1972). The two supernumerary branches have been identified as *either* one of pure graft and the other of pure host origin (Bullière) *or* both usually of dual origin (Bohn, Bart, French). Also, there have been suggestions that the results depend on *which* transverse axis is reversed (Bohn, 1972; French, 1976), and hence on the circumferential position from which the supernumeraries grow out.

It is interesting to look at the formation and structure of supernumerary leg regenerates in the cricket, *Acheta domesticus*, since the insect readily regenerates amputated legs, there are considerable differences between the cuticular structures on the different legs, and also grafts will take readily between *Acheta* and the much darker cricket, *Teleogryllus oceanicus* (French, in prep.).

The experiments presented here involve contralateral grafts between the tibiae of pro- and metathoracic legs, and between the metathoracic tibia and tarsus. Supernumerary branches are regenerated, as in similar experiments on the legs of other insects. The structure of these supernumeraries suggests that their composition *does* depend on their position: a supernumerary regenerated in an anterior or posterior position is reliably formed half from the host and half from the graft, while the origin of a medial or lateral supernumerary is variable. Some of these results have been briefly reported and discussed (French, 1982).

MATERIALS AND METHODS

Crickets (*Acheta domesticus*) were kept in large plastic containers at 25–26 °C and provided with ground dried cat food ('Go-Cat' – Carnation Pet Foods), damp cotton wool and occasional lettuce leaves. Adults were kept separately from the larvae and provided with pots of damp compost for egg laying. Amputations and grafts were performed on young animals (usually 4th–6th instar, 1 or 2 days after moulting) under CO_2 anaesthesia, using fine forceps and spring scissors. For grafting operations, animals of different instars were chosen, so that the donor leg segment was slightly smaller in diameter than the host leg segment. The graft was telescoped just inside the end of the stump and was held there by dried haemolymph. Operated animals were kept in plastic lunch boxes in groups of about 20 until their second or (usually) third postoperative moult, when the operated leg was removed, fixed in alcohol and examined.

<div align="center">RESULTS</div>

<div align="center">A. *Structure of the metathoracic and prothoracic legs*</div>

a) *Normal metathoracic leg*

Fig. 1A shows the tibia and tarsus of the normal metathoracic leg of *Acheta*. Notable cuticular features include the row of (usually six) large spines running down the tibia in anterior–lateral position, the row of (five or six) large posterior–lateral tibia spines, the characteristic group of six spines around the distal end of the tibia, the articulatory membrane on the lateral side of the tibia/tarsus joint, the double row of small spurs down the lateral face of the first tarsal segment, and the anterior and large posterior spine at the apex of the first tarsal segment.

b) *Regenerated metathoracic leg*

Metathoracic legs were amputated at proximal tibia level (the level used in grafting experiments) and 50 regenerates were examined after three post-operative moults. As shown in Fig. 1B, the regenerates reliably bore many of the normal cuticular markers including the two lateral rows of tibial spines (although rows contained two to ten spines), a characteristic pattern of four distal tibial spines, the articulatory membrane and both tarsal apical spines. However, one of the distal tibial spines was always missing and another was either missing or very small, and the lateral rows of tarsal spurs were always missing.

c) *Normal prothoracic leg*

Fig. 2A shows the normal prothoracic tibia and tarsus, which differ considerably in size and structure from those of the metathoracic leg. There are no lateral rows of spines down the tibia, no spurs on the tarsus and no apical tarsal spines. The tibia bears a very long bristle proximally on the lateral face and another on the posterior face, and has a characteristic group of three spines around its distal end. The first and second tarsal segments bear two rows of short, stout bristles down the medial face.

d) *Regenerated prothoracic leg*

Prothoracic legs were amputated in the proximal tibia and 51 regenerates were examined after three moults. The regenerates bore several of the normal markers (Fig. 2B) including two of the distal tibial spines and the medial tarsal bristles (although they were in two bands rather than precise rows). However, the long proximal lateral and posterior bristles were often missing or multiple and one of the distal tibial spines was either missing or very small.

Clearly, only those structures reliably formed on regenerated legs can be used as markers for graft or host origin of supernumerary legs regenerated from a graft/host junction. However, before grafting *between* the pro- and
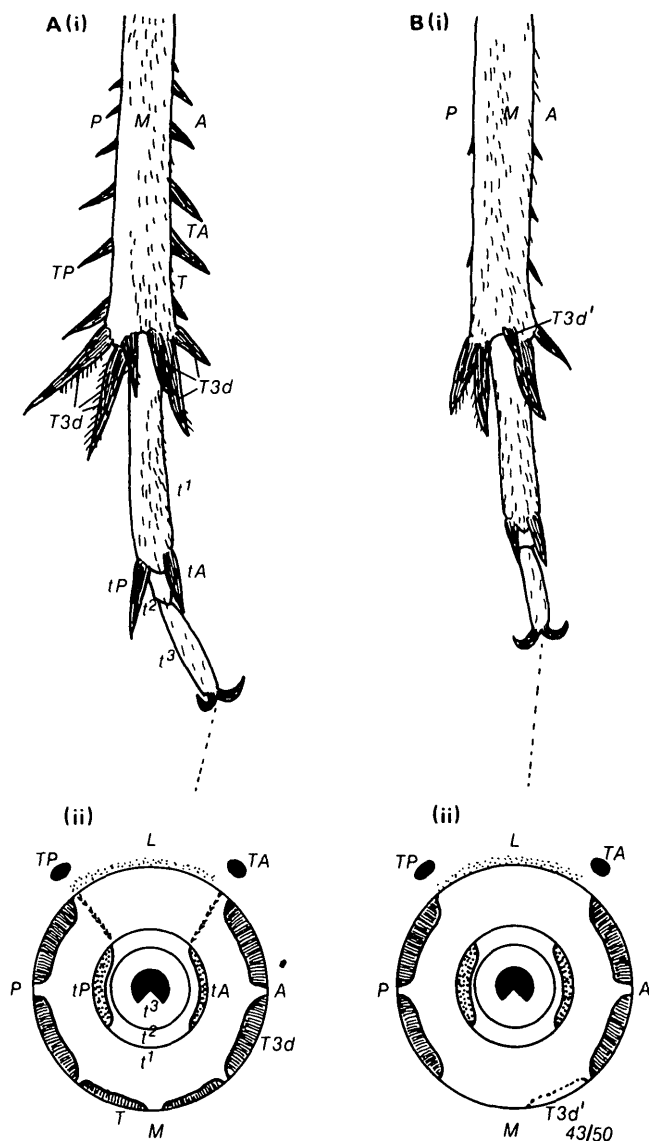
Fig. 1. Cuticular pattern on the tibia and tarsus of the metathoracic leg of *Acheta*. A, P, M, L, anterior, posterior, medial and lateral faces of the leg; T, tibia; t^1, t^2, t^3, first, second and third tarsal segments. (A) The normal metathoracic leg, shown in medial view (i) and in schematic 'end-on' view (ii). In (ii) the proximal levels are peripheral and the distal claws are in the centre, so the outer circle represents the articulation between tibia and first tarsal segment (with the extensive lateral articulatory membrane shown by stippling). The two rows of lateral tibial spines (TA and TP) are shown by solid ellipses, the bases of the six distal tibia spines ($T3d$) are hatched, and the bases of the two large spines at the apex of the first tarsal segment (tA and tP) are stippled. (B) The metathoracic leg regenerated from an amputation in the proximal tibia, shown in medial view (i) and in schematic 'end-on' view (ii). Note that one distal spine is always missing and another ($T3d'$ – shown by a dashed line in (ii)) is small and sometimes missing (present on 43 out of 50 regenerated legs). The normal leg has two rows of lateral tarsal spurs (shown by chevrons in A(ii)) which are missing from the regenerate.
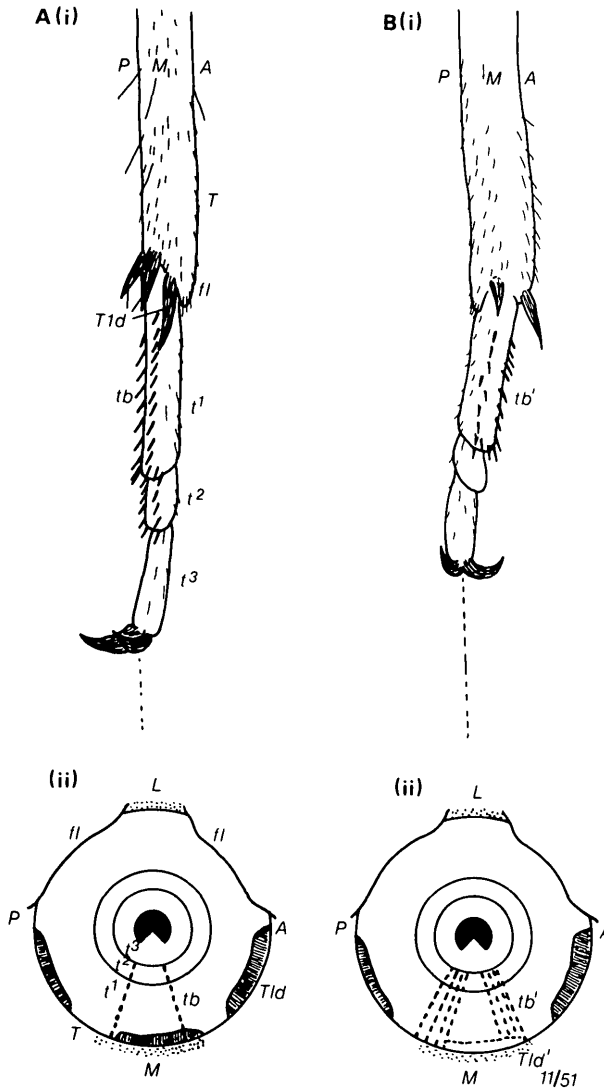
Fig. 2. Cuticular pattern on the tibia and tarsus of the prothoracic leg of *Acheta*. (A) The normal prothoracic leg, shown in anterior/medial view (i) and in schematic 'end-on' view (ii). In (ii) the outer circle represents the articulation between tibia and first tarsal segment, showing the prominent anterior and posterior flanges of cuticle (*fl*) and the articulatory membrane (stippled). The bases of the three distal tibial spines (*T1d*) are hatched and the two rows of stout bristles running down the medial face of first and second tarsal segments (*tb*) are shown by heavy dashed lines. (B) The prothoracic leg regenerated from an amputation in the proximal tibia, shown in posterior/medial view (i) and in schematic 'end-on' view (ii). Note that one distal tibial spine (*T1d'* – shown by a dashed line in (ii)) is small and sometimes missing (present on 11/51 regenerated legs). The medial tarsus has two bands of stout bristles (*tb'*), rather than the precise rows of the normal leg.
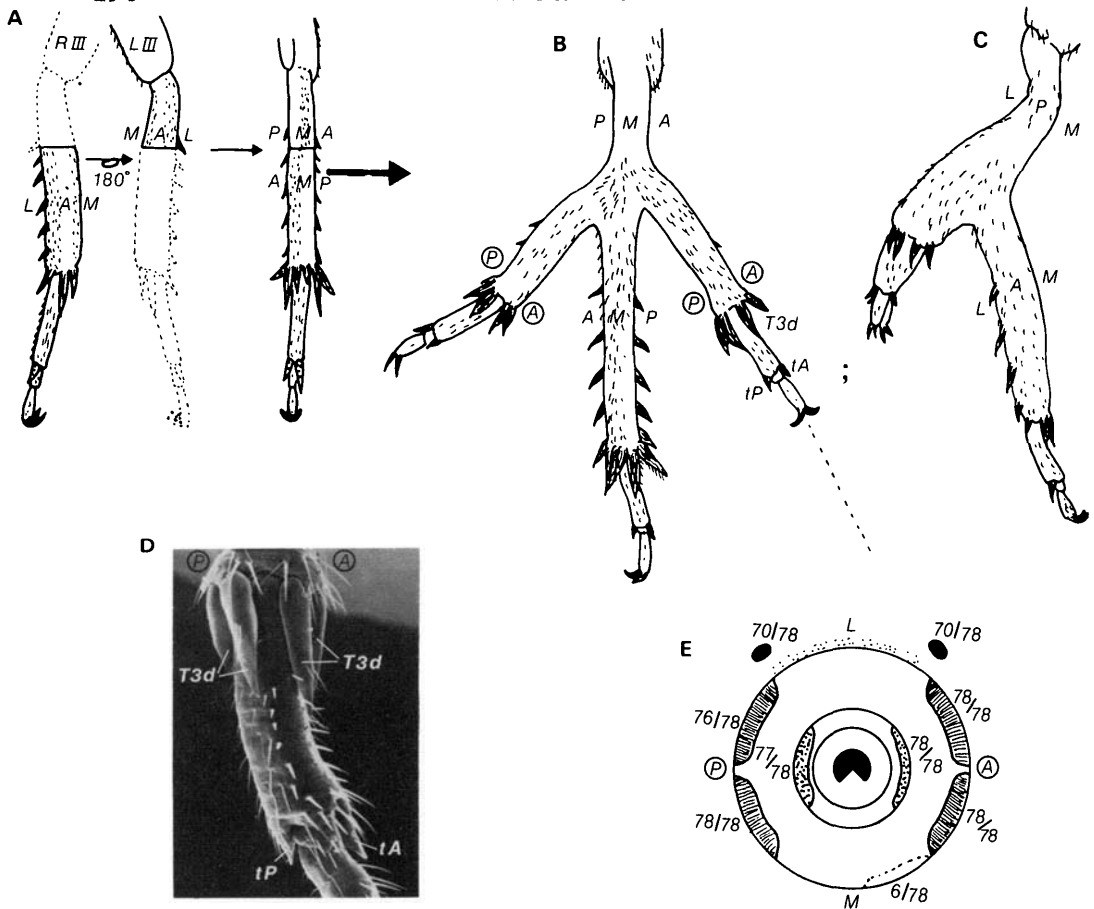
Fig. 3. Anterior–posterior axis reversal: metathoracic leg. (A) Schematic view of the operation, grafting the distal part of the right metathoracic leg (*RIII*) onto the stump of the left metathoracic leg (*LIII*), reversing the A/P axis. (B) Medial view of a resulting leg, showing separate supernumeraries in anterior (A) and posterior (P) positions. Anterior and posterior sides of supernumeraries are labelled Ⓐ and Ⓟ. (C) Posterior view of a resulting leg, showing a double fused supernumerary in lateral (L) position. (D) Scanning electron micrograph of the distal tibia and tarsus of a posterior supernumerary showing the four distal tibial spines (*T3d*) and the two apical tarsal spines (*tA* and *tP*). (E) Schematic 'end-on' view (see Fig. 1Bii) giving the occurrence of the particular cuticular structures on the 78 supernumeraries (from the 39 grafted legs).

metathoracic legs, I checked that the detailed structure of a supernumerary is, in fact, the same as that of a leg regenerated from an amputation site.

B. *Anterior–posterior axis reversal: metathoracic leg*

The right metathoracic leg of the donor was removed at proximal tibia level and grafted into the proximal tibia of the host left metathoracic leg, reversing the A/P axis of the graft (Fig. 3A). In all 39 successful cases the graft retained its structure (except for loss and regeneration of the tarsus in some cases) and its

orientation, and supernumerary structures were formed at proximal tibia level from the graft/host junction. In 37/39 cases separate supernumerary distal tibial and tarsal structures were regenerated in anterior and posterior positions (31 cases) or approximately anterior and posterior positions (6 cases). The supernumeraries were oriented like the host, with claws facing medially (Fig. 3B). In the remaining two cases a fused double supernumerary was formed in a lateral position with two sets of claws facing away from each other in the A/P plane
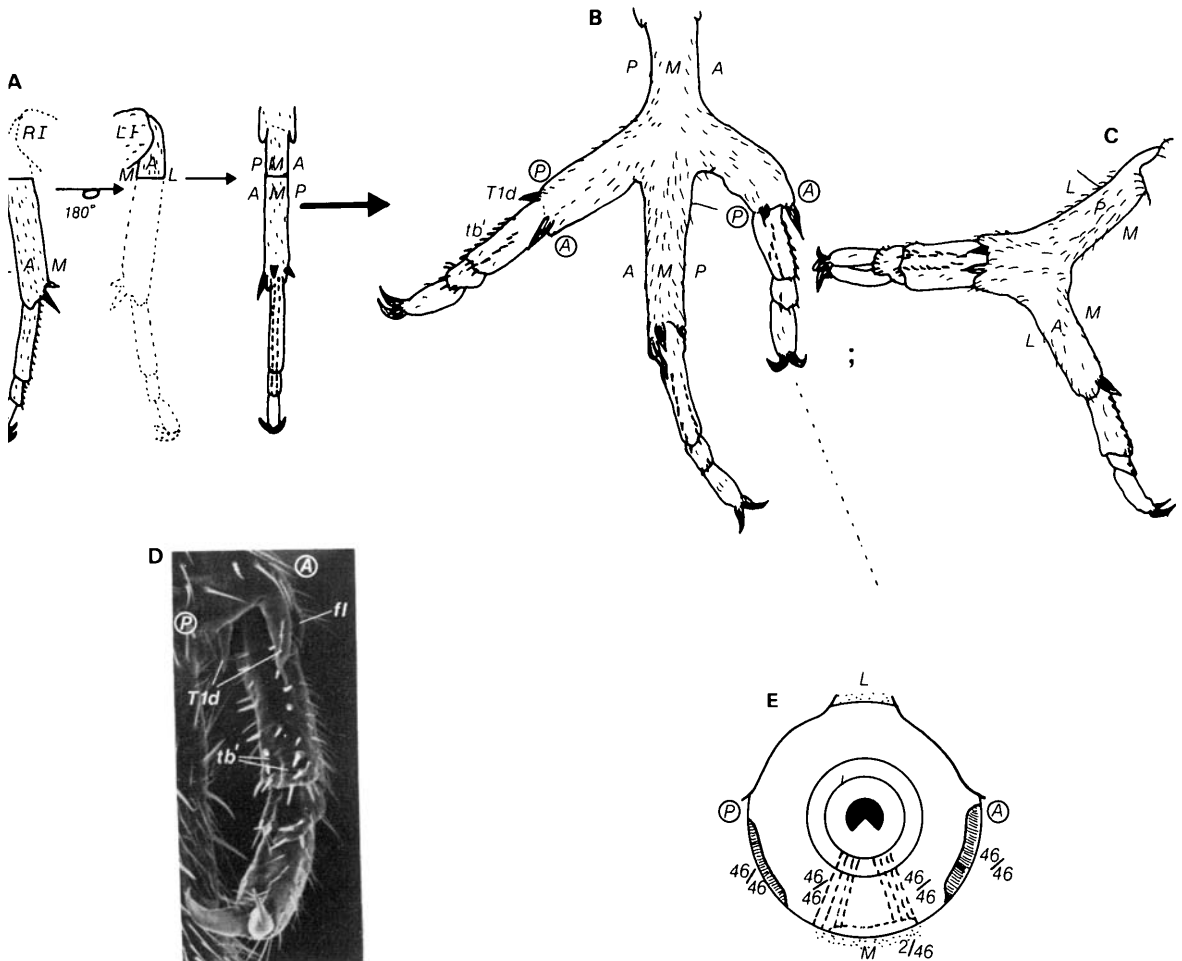

Fig. 4. Anterior–posterior axis reversal: prothoracic leg. (A) Schematic view of the operation, grafting the distal part of the right prothoracic leg (*RI*) onto the stump of the left prothoracic leg (*LI*), reversing the *A*/*P* axis. (B) Medial view of a resulting leg, showing separate supernumeraries in anterior (*A*) and posterior (*P*) positions. (C) Posterior view of a resulting leg, showing a double fused supernumerary in lateral (*L*) position. (D) Scanning electron micrograph of the distal tibia and tarsus of an anterior supernumerary, showing the anterior flange (*fl*), the two distal spines (*T1d*) and the bands of tarsal bristles (*tb'*). (E) Schematic 'end-on' view (see Fig. 2Bii) giving the occurrence of the particular cuticular structures on the 46 supernumeraries (from 23 scorable grafted legs).

(Fig. 3C). One of these double structures separated distally into two tarsi.

Of the total of 78 supernumeraries, most had two rows (or partial rows) of lateral tibial spines (Fig. 3E). Almost all of the supernumeraries had the metathoracic regenerate pattern of four distal spines, or four plus one small spine; only two legs had an abnormal pattern of only three apical spines. In all cases the lateral rows of tarsal spurs were absent. In all but one case the regenerates had prominent anterior and posterior tarsal apical spines (Fig. 3D, E).

It is evident that, apart from rarely forming the small fifth distal tibia spine and sometimes not forming the tibial lateral spines, the supernumeraries reliably have the cuticular structure of a regenerated metathoracic leg.

C. Anterior–posterior axis reversal: prothoracic leg

The right prothoracic donor leg was removed at proximal tibia level and grafted with A/P reversal into the proximal tibia of the host left prothoracic leg (Fig. 4A).

In 23/25 successful cases the graft retained its orientation, while in the other two cases it was rotated by 90° or less. In 15/25 cases graft and host had healed and regenerated supernumeraries from proximal tibia level while in the other cases the graft and host had regenerated independently to distal tibia or proximal tarsus level and then healed and formed supernumeraries.

In 10/25 cases separate supernumeraries were formed in approximately anterior and posterior positions, and were oriented like the host (Fig. 4B). In the other 15 cases a fused double structure (Fig. 4C) was formed in a lateral position (with claws facing away from each other in the A/P plane – 12 cases), or in a medial position (with claws facing away from each other in the M/L plane – three cases). Eleven of these double structures separated into two distal tarsi.

Of the total of 46 large analysable supernumeraries none bore lateral tibial spines, lateral tarsal spines or apical tarsal spines. All the supernumerary legs had the prothoracic regenerate pattern of two distal tibia spines or two plus the

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Fig. 5. Anterior–posterior reversal: graft between pro- and metathoracic legs. (A) Schematic view of the operation, grafting the distal part of the right prothoracic leg (*RI*) onto the stump of the left metathoracic leg (*LIII*), reversing the A/P axis. (B, C) Medial (B) and lateral (C) views of a resulting leg, showing separate supernumeraries in anterior (*A*) and posterior (*P*) positions. Each supernumerary has host structures (abbreviations as in Figs 1 and 3) on the host side, and graft structures (see Figs 2 and 4) on the graft side. (Di–iii) Detailed medial views of three *posterior* supernumeraries, showing the consistent half-and-half structure. In the detailed *camera lucida* drawings the lateral tibial spines and the claws are solid, the distal tibial spines are shaded, and the apical tarsal spines are stippled, as in the schematic 'end-on' diagrams. (Div) Schematic 'end-on' view of *posterior* supernumeraries showing approximate border between host (*h*) and graft (*g*) type structure, and the occurrence of particular cuticular structures on the 28 supernumeraries. (Ei–iii) Detailed medial views of three *anterior* supernumeraries. (Eiv) Schematic 'end-on' view of *anterior* supernumeraries.

third small spine (Fig. 4D, E). All supernumeraries had the two medial bands of stout bristles. Clearly, the supernumeraries reliably have the cuticular structures of a regenerated prothoracic leg. Hence the structure of a supernumerary formed after a graft between pro- and metathoracic legs should reveal whether it is formed from the graft, the host or both.
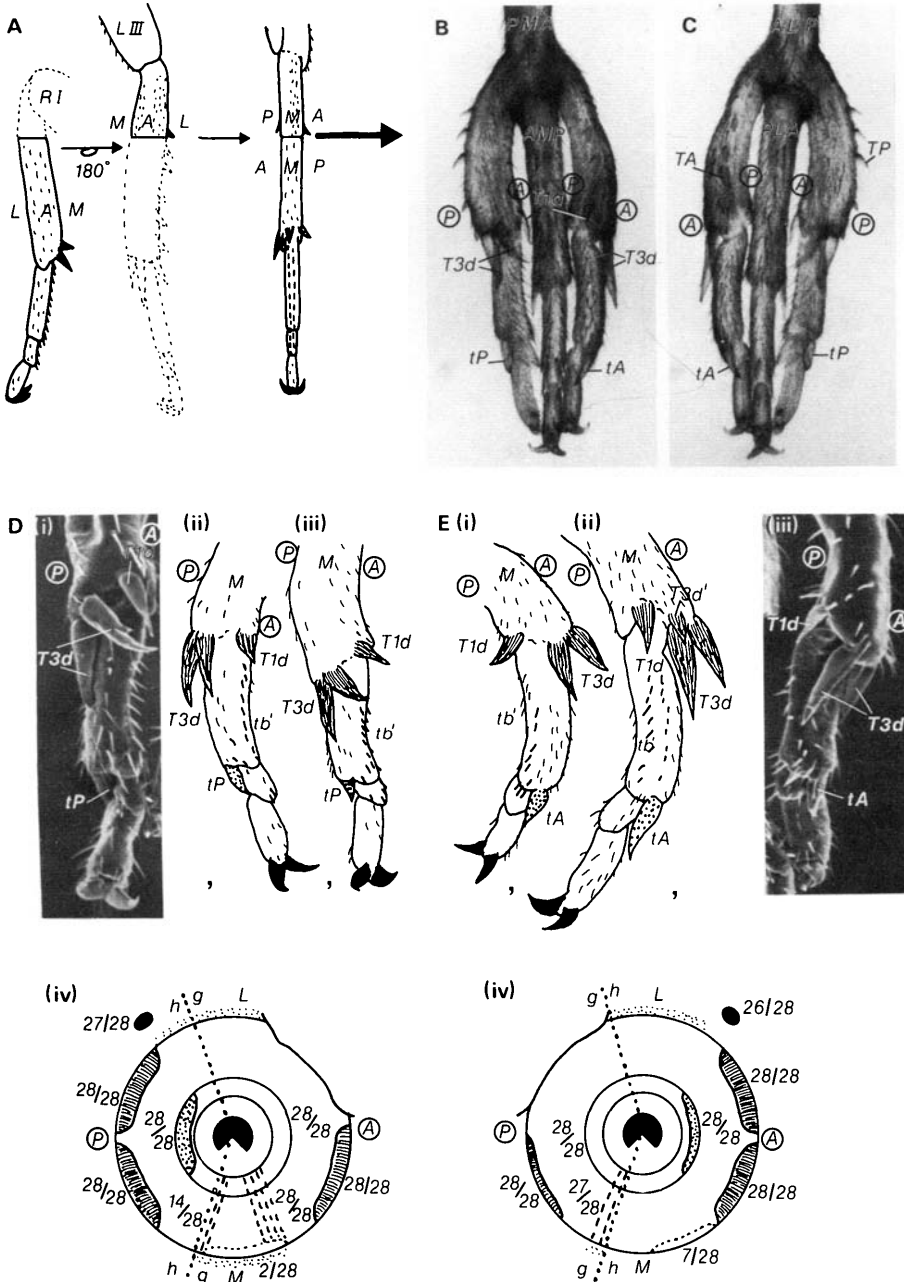
D. *Grafts between tibia of the pro- and metathoracic legs*

a) *Control orientation*

The left prothoracic donor leg was grafted at proximal tibia level without rotation into the tibia of the host left metathoracic leg.

In all 26 successful cases the graft retained its structure (except for loss and regeneration of the tarsus in some cases) and its orientation. No supernumerary structures were formed from the graft/host junction.

b) *Anterior–posterior axis reversal*

The right prothoracic donor leg was grafted at proximal tibia level onto the left metathoracic leg of the host, reversing the A/P axis of the graft (Fig. 5A).

In all 39 successful cases the graft retained its structure (except for loss and regeneration of the tarsus in some cases) and more or less its original orientation (i.e. the claws pointed medially with respect to the host leg). In 36 cases graft and host healed and formed supernumerary structures at proximal or mid tibial level (Fig. 5B), while in the other cases independent regeneration occurred to the distal tibia or first tarsal segment, at which level supernumeraries were formed.

In 28/39 cases two separate supernumeraries were formed, always at approximately anterior and posterior positions around the circumference of the graft/host junction (Fig. 5B, C). These supernumeraries were usually orientated like the host, with claws pointing medially (although in four cases the anterior supernumerary faced posteriorly and the posterior one faced anteriorly). The supernumeraries were remarkably *consistent in structure*, each forming host (metathoracic) structures on the host side and graft (prothoracic) structures on the graft side. Hence the *anterior supernumerary* (Fig. 5E) almost always formed one row of lateral tibial spines (only on the anterior side), the distal tibial spines were characteristic of the metathoracic leg on the anterior side and of the prothoracic leg on the posterior side, the first tarsal segment usually had some stout bristles down its posterior side and an apical spine only on its anterior side.

The *posterior supernumerary* (Fig. 5D) was precisely complementary in structure, forming metathoracic structures on its posterior side and prothoracic structures on its anterior side. The only variable feature involved the first tarsal segment, which appeared to have either an anterior medial band or both bands of stout bristles. However it was often difficult to distinguish these prothoracic bristles from the large bristles forming at the corresponding site on a metathoracic tarsus (Fig. 5Di, Eiii).

In the other 11 cases a fused double structure was formed in a lateral (six cases) or medial (five cases) position. The lateral supernumeraries (Fig. 6A) separated into two distal parts, positioned anteriorly and posteriorly, and orientated with claws pointing anteriorly and posteriorly respectively. Each of these distal parts was reliably half-host (metathoracic)/half-graft (prothoracic) in structure
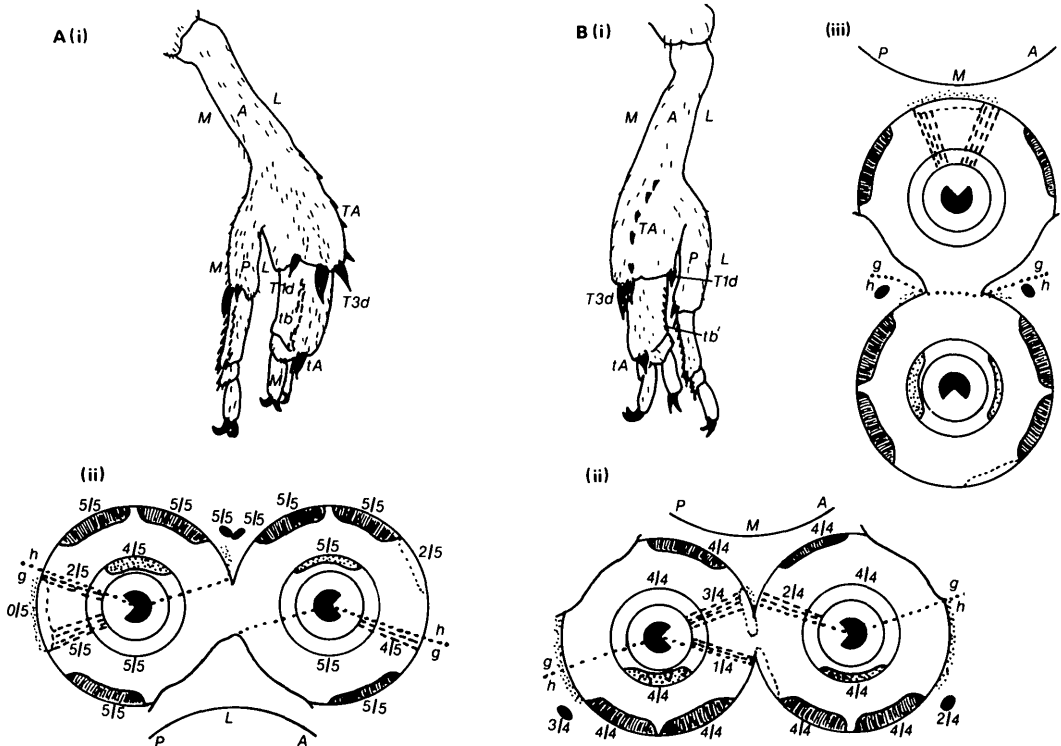
Fig. 6. Structure of fused double supernumeraries resulting from A/P reversed grafts between pro- and metathoracic legs. (A) Fused supernumeraries in *lateral* position (6/11 cases), shown in anterior view (i) and in schematic 'end-on' view (ii). Each component leg of the double structure is half-and-half in structure, and Aii gives the occurrence of structures on the five analysable legs. (B) Fused supernumeraries in *medial* position (5/11 cases), shown in anterior view (i) and in schematic 'end-on' view (ii, iii). The component legs either had claws facing towards each other (4/5) and were half-and-half in structure (ii), or had claws facing away from each other and were one of host type and the other of graft type (i, iii).

(Fig. 6C). In four out of five cases the medial supernumeraries separated into anterior and posterior distal parts, orientated facing posteriorly and anteriorly respectively, and each half-host/half-graft in structure (Fig. Bii). In the other case the medial supernumerary had medial and lateral distal parts, orientated facing medially and laterally respectively, and of completely host and graft structure respectively (Fig. Bi, iii).

c) *Medial–lateral axis reversal*

The right prothoracic donor leg was grafted at proximal tibia level onto the left metathoracic leg of the host, reversing the M/L axis of the graft (Fig. 7A).

In 68 of the 76 successful cases the graft retained its structure (except for loss and regeneration of the tarsus in some cases) and more or less its original orientation: in the other eight cases the graft had become misaligned and these will not

be considered. In 67 of the 68 cases graft and host healed and formed super-
numerary structures at proximal or mid tibial level, while in the remaining case
they regenerated independently to the first tarsal segment, where super-
numeraries were formed.

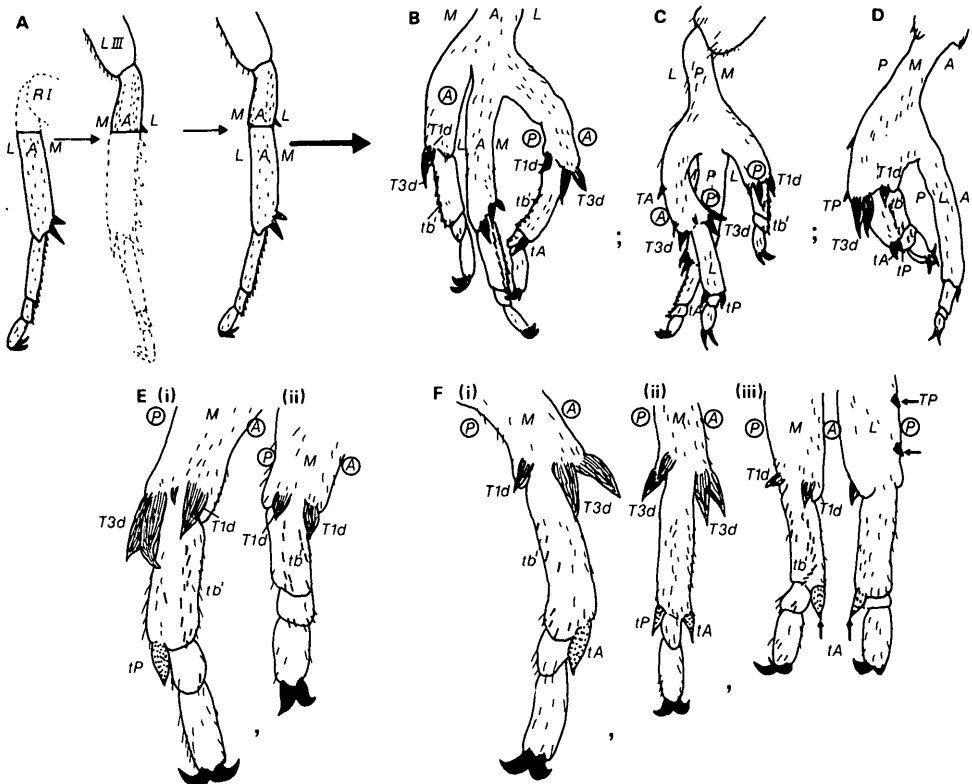In 44 of the 68 cases two separate supernumeraries were formed, at medial and

Fig. 7. Medial–lateral axis reversal: graft between pro- and metathoracic legs. (A)
Schematic view of the operation, grafting the distal part of the right prothoracic leg
(*RI*) onto the stump of the left metathoracic leg (*LIII*), reversing the M/L axis. (B,
C) Anterior (B) and posterior (C) views of two resulting legs, showing separate
supernumeraries in medial (*M*) and lateral (*L*) positions. The leg shown in (B) has
supernumeraries which are each half-and-half in structure, while the leg in (C) has
one prothoracic and one metathoracic supernumerary. (D) Medial view of a result-
ing leg, showing a double fused supernumerary in posterior (*P*) position. (Ei, ii)
Detailed medial views of two *medial* supernumeraries, showing variability in struc-
ture. Ei is anterior-graft/posterior-host and Eii is pure graft in structure. (Fi–iii)
Medial (i, ii) or medial and lateral (iii) views of three *lateral* supernumeraries,
showing variability in structure. Fi is posterior-graft/anterior-host and Fii is pure
host in structure. Eiii is largely of graft type but has some host-type structures
(arrowed). (G) 'End-on' views of the three categories of *medial* supernumerary: Gi
– *graft* type (18/41 analysable cases, see Eii), Gii – *posterior-host/anterior-graft*
(18/41, see Ei), and *variable* (5/41 – one example shown in Giii). (H) Schematic
'end-on' views of the three categories of *lateral* supernumerary: Hi – *host* type (6/39
analysable cases, see Fii), Hii – *anterior-host/posterior-graft* (25/39, see Fi), and
variable (8/39 – examples shown in Hiii and in Fiii).

lateral (24 cases) or approximately medial and lateral (20 cases) positions on the graft/host junction (Fig. 7B). The supernumeraries were usually orientated with claws pointing medially (although a few of the medial supernumeraries faced posteriorly and a few of the lateral supernumeraries faced anteriorly).

These supernumeraries, in marked contrast to those derived from the A/P reversal, were very *variable in structure* in a way which did not seem to depend on slight differences in their position of origin or orientation. The *medial super-numerary* (Fig. 7E, G) was either (i) completely graft (prothoracic) in structure, (ii) of graft structure on the anterior side and host structure on the posterior side, with borders falling in the *same* positions as on the A/P supernumeraries, or (iii) of mixed graft and host structure with the borders falling in *different and variable* positions. The *lateral supernumerary* (Fig. 7F, H) was either (i) completely host (metathoracic) in structure, (ii) of host structure on the anterior side and graft structure on the posterior side, or (iii) of mixed structure with borders in *different*
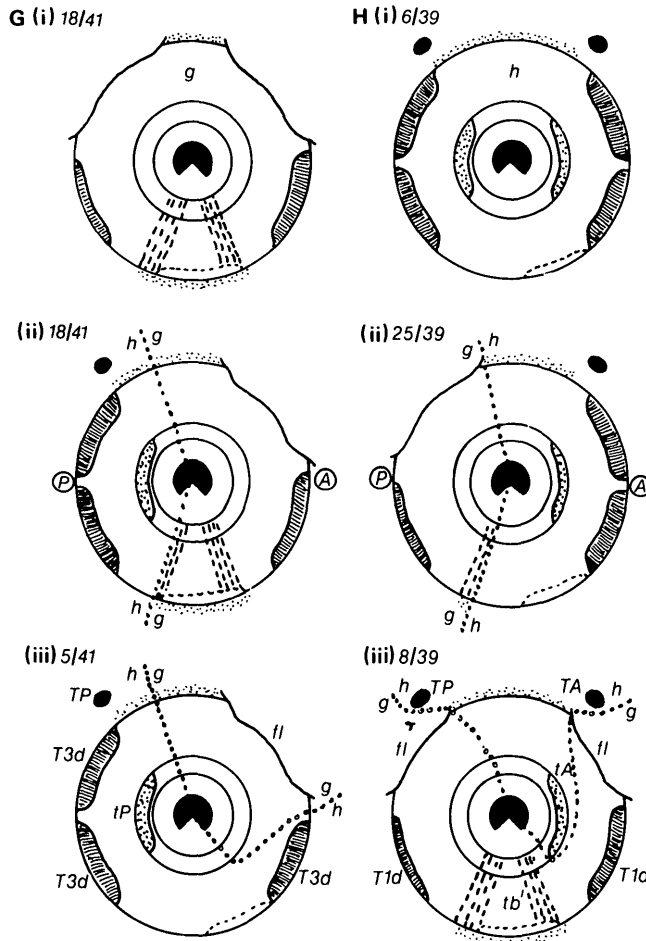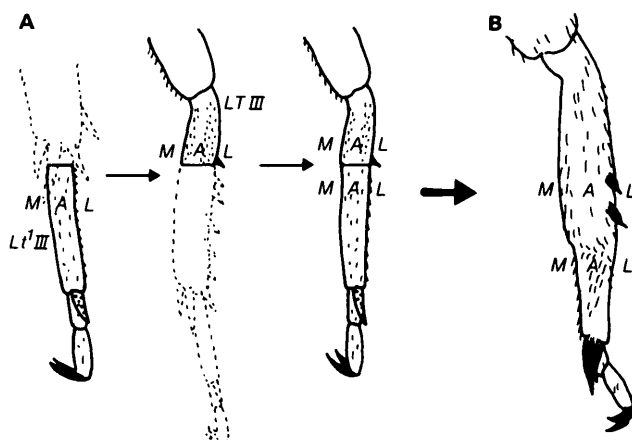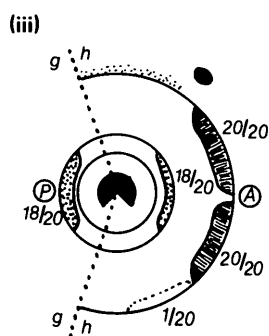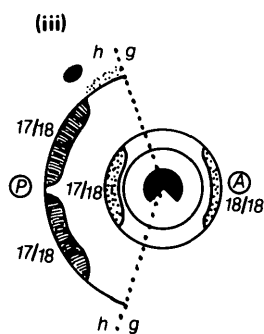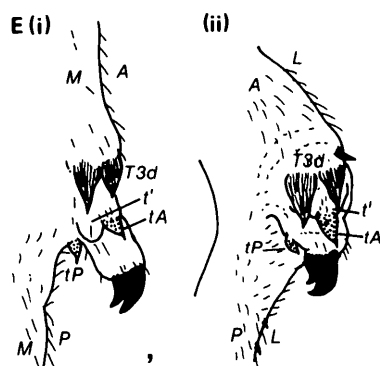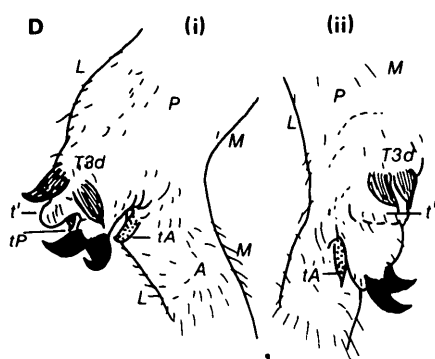

Fig. 7b

Fig. 8. Control graft between tarsus and tibia of the metathoracic leg. (A) Schematic view of the operation, grafting the distal part of the left tarsus (*Lt^l III*) onto the stump of the left tibia (*LT III*). (B) Anterior view of a resulting leg.

and variable positions. Hence, unlike the A/P supernumeraries, the M/L super-numeraries were variable in structure and the two supernumeraries on a given leg often did *not* form complementary structures (e.g. the medial supernumerary could be of completely graft structure while the lateral supernumerary was anterior-half-host/posterior-half-graft in structure).

In the remaining 24 out of 68 cases a fused double structure was formed in a posterior position (Fig. 7D). Most of these structures separated distally into two component tarsi which were positioned *either* (i) medially (orientated towards medial, medial/posterior or posterior) and laterally (orientated towards lateral, lateral/anterior or anterior) (21 out of 24 cases), *or* (ii) posteriorly (orientated towards anterior or medial) and anteriorly (orientated towards posterior or lateral) (3 out of 24 cases). The composition of these 48

Fig. 9. Anterior–posterior axis reversal: graft between metathoracic tibia and tarsus. (A) Schematic view of the operation, grafting the distal part of the right tarsus (*Rt^l III*) onto the stump of the left tibia (*LT III*), reversing the A/P axis. (B) Medial/anterior view of a resulting leg, showing separate supernumeraries in anterior (*A*) and posterior (*P*) positions. Each supernumerary has structures distal to the host level on the host side, and structures distal to the graft level on the graft side. (C) Anterior view of a resulting leg with a double fused supernumerary in medial (*M*) position. (Di, ii) Detailed views of two *posterior* supernumeraries. In Dii the apical tarsal spine (*tA*) is evident on the graft side of the supernumerary, while the host side shows two distal tibial spines (*T3d*) and a lobe (*tl*) but no tarsal spine (such as can be seen on Di). (Diii) Schematic 'end-on' view of the *posterior* supernumeraries showing the approximate border between host (*h*) and graft (*g*) level structures, and the occurrence of particular structures on the 18 scorable supernumeraries. It was not possible to decide whether lateral tibial spines (*TA* and *TP*) were part of the host leg or the supernumerary. (Ei, ii) Detailed views of two *anterior* supernumeraries, showing the consistent half-and-half structure. (Eiii) Schematic 'end-on' view of the *anterior* supernumeraries.

supernumerary distal structures was variable in a way which did not depend on their precise position or orientation. They were either (i) completely graft (prothoracic) or completely host (metathoracic) in structure (12 out of 40 scorable structures), or (ii) anterior-half/posterior-half in structure (15 out of 40), or (iii) of mixed structure with borders in various different positions

(13 out of 40). Furthermore, as was the case for the two separate M/L super-numeraries, the two component parts of a double supernumerary often did *not* form complementary structures.

In view of the striking difference between the composition of super-numeraries resulting from the A/P and the M/L axis reversals, these grafts were repeated between the tibia and the first tarsal segment of the metathoracic leg.
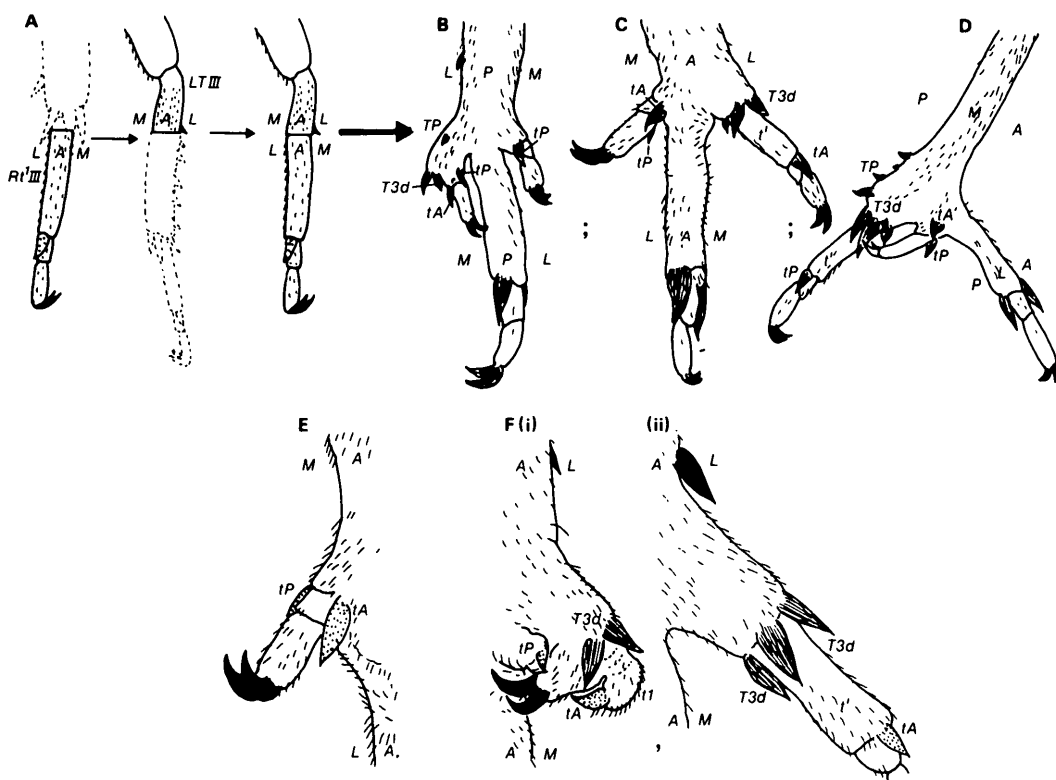

Fig. 10. Medial–lateral axis reversal: graft between metathoracic tibia and tarsus. (A) Schematic view of the operation, grafting the distal part of the right tarsus (*Rt^l III*) onto the stump of the left tibia (*LT III*), reversing the *M/L* axis. (B, C) Posterior (B) and anterior (C) views of resulting legs showing separate super-numeraries in medial (*M*) and lateral (*L*) positions. The leg shown in (B) has one graft level supernumerary and one half-and-half in structure, while the leg in (C) has one graft level and one host level supernumerary. (D) Medial view of a resulting leg with a supernumerary which is fused at the base but separates into two free distal ends. (E) Detailed view of a *medial* supernumerary of graft-level structure. (Fi, ii) Detailed views of two *lateral* supernumeraries, one of host level structure and the other half-and-half in structure. (G) Schematic 'end-on' views of the three categories of *medial* supernumerary: Gi – *graft* level (28/30 analysable cases, see E), Gii – *posterior-host/anterior-graft* (1 case), and *variable* (1 case, shown in Giii). (H) Schematic 'end-on' views of the three categories of *lateral* supernumerary: Hi – *host* level (16/27 analysable cases, see Fii), Hii – *anterior-host/posterior-graft* (7/27, see Fi), and *variable* (4/27 – one example shown in Hiii).

E. *Grafts between the tibia and first tarsal segment of the metathoracic leg*

a) *Control orientation*

The donor left metathoracic leg was amputated at the proximal level in the first tarsal segment and the distal part was grafted without rotation into the proximal tibia of the host left metathoracic leg (Fig. 8A).

In all 25 successful cases the graft retained its structure and orientation. In 24 cases graft and host healed without forming any supernumerary structures (Fig. 8B). In the remaining case graft and host had regenerated independently to the distal level of their respective segments and had then healed and formed a supernumerary outgrowth bearing two spines.

b) *Anterior–posterior axis reversal*

The donor right metathoracic leg was amputated at the proximal level in the
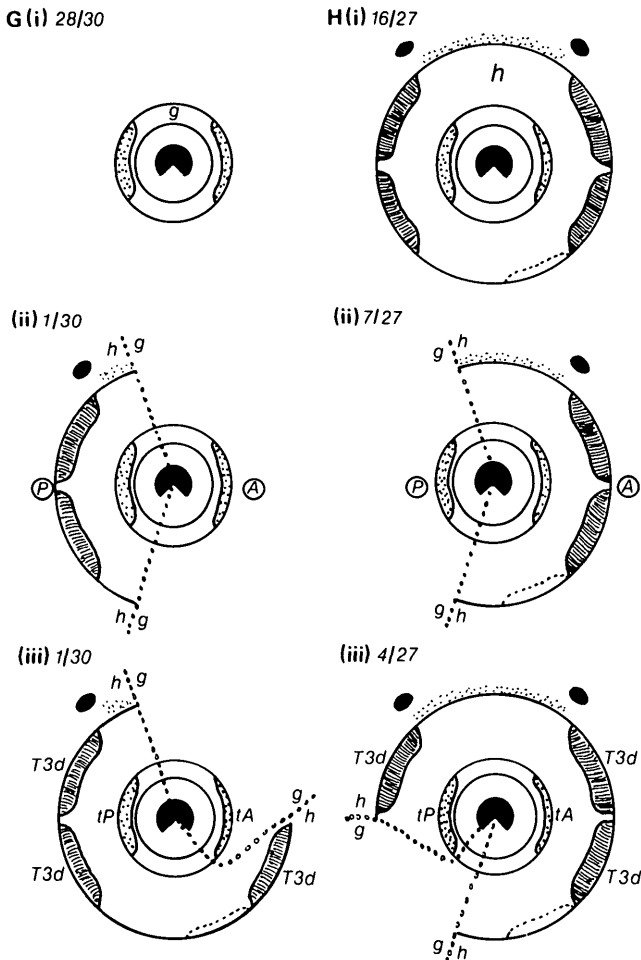

Fig. 10b.

first tarsal segment and grafted A/P reversed into the proximal tibia of the host left metathoracic leg (Fig. 9A).

In 52 out of 55 successful cases the graft retained its structure and original orientation: in the other three cases the graft had become misaligned and these will not be considered further.

In 25 out of 52 cases the graft and host regenerated independently to the level of the first, the second or the third tarsal segment, where they healed and generated supernumerary structures. These were either two separate super- numeraries (16 out of the 25 were in anterior and posterior positions and orien- tated facing medially) or a fused double structure (in a medial (six cases) or lateral (three cases) position with sets of claws facing away from or towards each other in the A/P plane). These supernumerary structures are just distal parts of the metathoracic tarsus and hence give no information about their graft or host origin.

In the remaining 27 cases supernumeraries formed from the junction between the host tibia and the graft tarsus, and hence their structure is informative. 23 out of 27 of these legs bore two separate supernumeraries, in approximately anterior and posterior positions (Fig. 9B). The structure of these super- numeraries was remarkably *consistent*. The *anterior supernumeraries* had a bulg- ing anterior partial tibial apex in addition to the tarsal structures (Fig. 9E), while the *posterior supernumerary* had a bulging posterior partial tibial apex (Fig. 9D). Thus, as in the case of the prothoracic/metathoracic tibia grafts, an A or P supernumerary has, on the host side, the structures which would be regenerated from the host amputation surface and, on the graft side, structures which the graft surface would regenerate.

The other four legs bore fused double structures (Fig. 9C) in medial or anterior positions but, since only two were well developed, they will not be analysed in detail.

c) *Medial–lateral axis reversal*

The donor right metathoracic leg was amputated at the proximal level of the first tarsal segment and grafted M/L reversed into the proximal tibia of the host left metathoracic leg (Fig. 10A).

In 43 of the 51 successful cases the graft retained its structure and original orientation; in the other eight cases the graft was misaligned and these will not be analysed.

In five of the 43 cases the graft and host regenerated independently to the level of the first or second tarsal segment, healed and formed supernumerary struc- tures. These either consisted of two separate supernumeraries (four out of five were in approximately medial and lateral positions and orientated approximately medially) or a fused double structure (one in an anterior position with sets of claws facing away from each other in the M/L plane). The supernumerary structures were just distal parts of the tarsus, and therefore are not informative

as to their origin from the graft or host component of the grafted leg.

The other 38 out of 43 legs had supernumeraries forming from the junction between host tibia and graft tarsus. 33 of these legs had two separate supernumeraries at medial (or approximately medial) and lateral (or approximately lateral) positions (Fig. 10B, C). Supernumeraries were usually orientated more or less medially.

The separate M/L supernumeraries, unlike the A/P supernumeraries but like the prothoracic/metathoracic tibia M/L supernumeraries, were *variable in structure*. The *medial supernumerary* (Fig. 10E, G) consisted of either (i) structures distal to the graft (i.e. first tarsal segment) level, (ii) structures distal to the graft on the anterior side and those distal to the host on the posterior side, or (iii) of mixed structure but with the border in a different position. The *lateral supernumerary* (Fig. 10F, H) consisted of either (i) structures distal to the host (i.e. tibia) level (ii), structures distal to the host on the anterior side and those distal to the graft on the posterior side, or (iii) of mixed structure but with the borders in different and variable positions. Thus these supernumeraries (like the prothoracic/metathoracic tibia M/L supernumeraries but unlike both kinds of A/P supernumeraries) were variable in structure, being either totally graft or host type in structure, or anterior half/posterior half in structure with borders in the same positions as in the A/P supernumeraries, or of mixed structure with borders in a variety of positions. The two supernumeraries on a given leg often (10 out of 33 cases) did *not* form complementary structures.

The remaining legs formed fused double supernumeraries (Fig. 10D) in a posterior (three of five) or an anterior (two of five) position, and their component tarsal structures were positioned and orientated in several different ways. Scoring these fused component tarsi was difficult, but it seemed that they could be either (a) of graft or host regenerate structure (seven out of ten) or (b) anterior-half/posterior-half in structure (three out of ten). In one case the two component parts of a double supernumerary clearly formed non-complementary structures.

DISCUSSION

It has been known for a long time that grafting the leg of a cockroach (Bohn, 1965) or stick insect (Bart, 1965) or beetle (Balazuc, 1948) onto the contralateral leg stump, causes a supernumerary leg of host handedness and orientation to form at each of the two points of maximum positional discrepancy on the graft/host junction. Sometimes these supernumeraries fuse to form one double structure arising in an intermediate position. The present experiments extend this finding to the cricket, and they also show (as has previous work on cockroach and stick insect legs) that typical supernumeraries form after grafts between homologous segments of pro- and metathoracic legs, and between different segments of the metathoracic leg.

Metameric segment type is determined very early in the embryonic development of insect epidermis, and is a stable cellular property. It changes only in exceptional circumstances, such as the heteromorphic regeneration of leg tarsus from proximal levels of the stick insect antenna (Urvoy, 1970) or the transformation of *Drosophila* imaginal disc cells in homoeotic mutants or after prolonged *in vivo* culture. There is no indication that metameric determination of insect leg epidermis can change after grafting, so the detailed structure of a supernumerary arising from a prothoracic/metathoracic leg graft can be used to analyse which parts are formed from graft and which from host tissue. Similarly, regeneration from an amputation site or a graft/host junction produces all structures distal to that level, so the structures present on a supernumerary regenerated from a tibia/tarsus graft indicate whether it arises from graft, host or both.

There have been several suggestions concerning the formation of these supernumerary legs and their relationship to normal development. Hence they have been attributed to the *isolation* of the differently orientated graft and host surfaces (Bullière, 1970), to the *interaction* between graft and host cells from opposite faces of the limb (Bart, 1971) or to the pattern of *intercalary regeneration* between the graft and host cells interacting at the junction (French, 1976). Since all of these ideas can account for the number, position and orientation of supernumeraries regenerated after a left/right graft, knowledge of the composition (graft or host origin) of the supernumeraries is vital to any attempt to understand how they form.

There have been several previous attempts to determine the composition of supernumerary regenerates. Bullière (1970) grafted at coxa and at tibia level between pro- and metathoracic legs of the cockroach, *Blabera*. He concluded that the supernumeraries formed after A/P or M/L reversal were *always* one of pure host type and one of pure graft type. The legs differ in the relative size of their segments but, since the supernumeraries are often poorly formed, the only unequivocal marker was the presence (prothoracic) or absence (metathoracic) of spines in the row of anterior–medial bristles on the femur. Bohn (1972) performed similar grafts between pro- and metathoracic legs of the cockroach, *Leucophaea*, and also grafted between *Leucophaea* and the darker species, *Gromphadorhina*. He concluded that supernumeraries were usually of *mixed* origin, and I reached a similar conclusion (French, 1976) after tibia level grafts between different cuticle colour mutants of *Blattella*. Bart (1971) produced supernumerary legs in the stick insect, *Carausius*, by grafting strips between A and P and between M and L faces of the pro-, meso- and metathoracic legs. There were few morphological markers, but Bart concluded that supernumeraries were usually of mixed composition. The present results from grafting between pro- and metathoracic cricket legs, and between metathoracic tibia and tarsus, lead to rather different conclusions: after *A/P* reversal supernumeraries are *always* anterior-half/posterior-half in composition, while *M/L* supernumeraries are *variable* but are usually of mixed composition.

The cricket supernumerary legs formed after A/P reversal are invariant in composition. Judging from the presence of pro- or metathoracic structures down the lateral face and around the distal end of the tibia, and down the medial face and at the apex of the first tarsal segment, the anterior half of the anterior supernumerary and the posterior half of the posterior supernumerary *always* derive from the host. The other two complementary halves are *always* formed from the graft. The same conclusion follows from the presence or absence of tibia structures on supernumeraries resulting from grafts between tibia and tarsus. The boundaries between host- and graft-derived parts seem to lie in constant positions on the lateral and medial faces of the supernumeraries (Figs 5, 9). Bullière (1970) scored 29 legs after A/P reversal at the coxa level and found, in 23 cases, that the anterior supernumerary was 'host' while the posterior one was 'graft' in structure. Since his major marker was an *anterior* structure, his results are compatible with each supernumerary being half-and-half in structure (see also Bohn, 1972, pg. 202). Bohn's results from interspecies cockroach grafts show that A/P supernumeraries are usually approximately half-and-half in origin but he did *not* find that the boundaries were always in precisely the same positions (see Bohn, 1972, figs 2 and 6).

The cricket legs formed after M/L reversal are variable in composition. The medial supernumerary may be either pure graft (but never pure host), or anterior-half-graft/posterior-half-host (but never *vice versa*), or variable in structure. The lateral supernumerary, on the other hand, may be either pure host, or anterior-half-host/posterior-half-graft, or variable in structure. These categories of results are found after both the prothoracic/metathoracic and tibia/tarsus grafts (Figs 7, 10) and the two supernumeraries on a given leg are frequently not complementary in structure. Bullière (1970) scored 26 legs after M/L reversal at the coxa level and found, in 20 cases, that the medial supernumerary was 'graft' while the lateral one was 'host'. Because his marker was anterior, these results are compatible with the 'pure' and the 'half-and-half' categories of cricket supernumerary legs. However, Bohn's (1972) results from M/L reversed cockroach interspecies grafts certainly do differ from the cricket leg results. He found that the lateral supernumerary could be of pure host (Bohn, 1972, fig. 3E) or *pure graft*. (Bohn, 1972, fig. 8F) origin, and that, whilst most supernumeraries were of mixed origin, there was *no* sign of a major category of anterior-half/posterior-half supernumeraries (Bohn, 1972, figs 3 and 8).

The recent Polar Co-ordinate model, or PCM (French *et al.* 1976; Bryant *et al.* 1981), links the formation of supernumeraries to intercalary regeneration. If a strip of the epidermis and cuticle is moved around the circumference of the femur of the cockroach leg and grafted into a different position, normally non-adjacent cells are confronted at the graft/host junction. This stimulates local growth and intercalary regeneration, forming the section of circumference which normally separates the original graft and host positions, by the *shortest route* (French, 1978). If strip grafts are made between segments with different cuticular

structures, then the structure of the intercalary regenerate shows that it is formed from both edges of the junction. However, in a wide range of different graft combinations, the border between graft- and host-derived parts of the regenerate lies in a position just posterior to mid-medial (for intercalation going via the medial route around the circumference) or just posterior to mid-lateral (for an intercalary regenerate of lateral tissue). This suggests that the leg consists of anterior and posterior compartments with borders which cannot be crossed during intercalation (French, 1980). After a left/right graft, similar intercalation will occur at sites on the junction where confronted graft and host cells come from different positions, and this will create two complete circumferences (Fig. 11A). These will act as bases for distal regeneration (just like the circumference at an amputation site), so two supernumeraries will form. Does this model make any predictions about the composition of these supernumeraries?

If the cricket leg is divided into two compartments with borders in the same positions as those of the cockroach leg, then the complete circumferences generated by A/P reversal will be at positions well away from the borders. Graft and host will each intercalate up to the borders and therefore the circumferences will be half-and-half in composition, and the boundaries between host-type and graft-type structure will run down the resulting supernumeraries in the positions of the compartment borders (Fig. 11A). After M/L reversal, intercalation of the circumferences will occur in lateral and medial positions and may thus start from tissue which spans the borders. In this case intercalation will not be reliably restricted at any position. Graft and host will contribute variable amounts to the circumferences, with the boundaries between them not corresponding to compartment borders (Fig. 11B).

Fig. 11. Polar co-ordinate model interpretation of the formation of cricket supernumerary legs. The host circumference (h) is represented by the outer circles, the graft (g) by the inner circles and between these are the bases of the two supernumeraries. Graft and graft-derived tissues are stippled. Position around the circumference is marked by positional values *1–12* (in A) and the borders between anterior and posterior compartments are marked by heavy lines in positions $5\frac{1}{2}$ and $\frac{1}{2}$. (A) Anterior–posterior axis reversal. Confrontation of dissimilar graft and host cells at the junction will lead to intercalary regeneration by the shortest route (e.g. at the point where cells *2* and *10* interact, cells with values *1, 12, 11* will be intercalated). This process will lead to the formation of complete circumferences in anterior ('A') and posterior ('P') positions. Throughout this process intercalation will be restricted at the border positions, so each supernumerary circumference (and subsequent supernumerary leg) will be half-graft/half-host in origin. (B) Medial–lateral axis reversal. Intercalation between graft and host will generate extra circumferences at medial ('M') and lateral ('L') positions. Since these positions may span the compartment borders, intercalation may not be restricted at any particular positions, so the graft and host may be expected to contribute in variable proportions to the supernumeraries (Bi). However, this does *not* explain why a medial supernumerary is often of pure graft origin (as in Bii) while a lateral one is often of pure host origin, or why a medial supernumerary is often anterior-half-host/posterior-half-graft while a lateral one has the opposite structure (as in Bii).

This interpretation is based on the general result that A/P supernumeraries are reliably half-and-half while the M/L supernumeraries are variable in composition. This general result is also found after comparable grafting between pro- and metathoracic legs in *Tenebrio* (French, in prep.) but, as mentioned above, it is not in complete agreement with the results of Bohn (1972) who grafted between differently pigmented cockroaches. In experiments where the markers for graft or host origin consist of the presence or absence of cuticular structures (e.g. the tarsal apical spine – Figs 5, 7), it is not possible to determine the *exact* position of the boundary. In addition, the marker may consist of the number or arrangement of structures, rather than one defined structure (e.g. the distal tibia spines – Figs 5, 7), so scoring depends on interpretation. For these reasons, these and other grafts have been repeated between *Acheta* and the darker cricket, *Teleogryllus*, where a distinct boundary can usually be traced between the light and dark cuticle: again, the A/P supernumeraries are always half-and-half, while
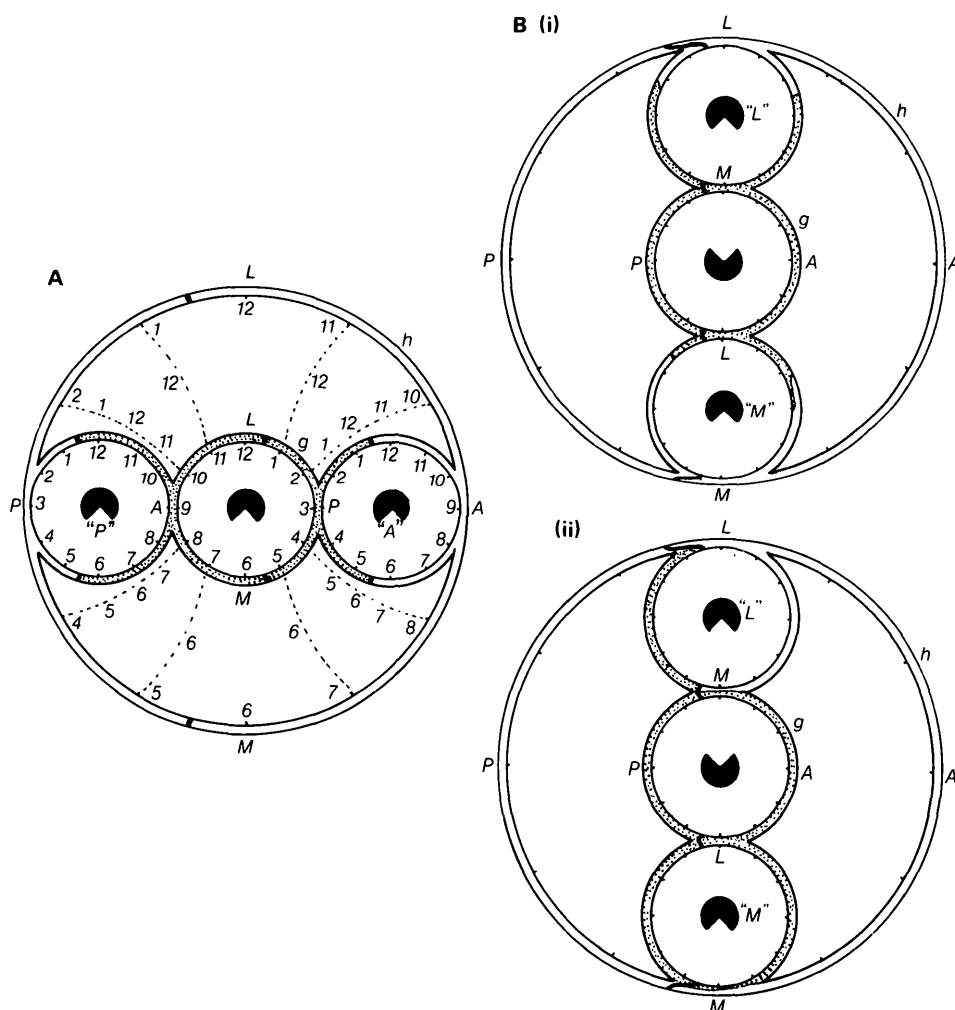
supernumeraries originating elsewhere are of variable origin (French, 1982 and in prep.).

However, the PCM and stable compartments really only explain one category of the cricket M/L supernumerary legs: the other categories are not satisfactorily explained. Hence, although the occasional pure graft or pure host supernumerary would be expected, there is *no* prediction that they would be a major category of result, or that medial ones would only be pure graft and lateral ones only pure host. Similarly, the occasional mixed-composition supernumerary might form with anterior and posterior compartments of different origin, but there is *no* prediction that they would frequently occur, or that a medial one would always be anterior-graft/posterior-host and a lateral one would always be the converse (Fig. 11C). These results would require extra *ad hoc* assumptions (about different rates of intercalation and exact positions of borders) for which there is, at present, no evidence. Clearly, despite the length of time over which developmental biologists have been constructing supernumerary legs in various insect species, the number of studies of their structure and composition, and the number of models purporting to explain them, we do not yet fully understand their formation.

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