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Joint morphology in the insect leg: evolutionary history inferred from *Notch* loss-of-function phenotypes in *Drosophila*

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SUMMARY

Joints permit efficient locomotion, especially among animals with a rigid skeleton. Joint morphologies vary in the body of individual animals, and the shapes of homologous joints often differ across species. The diverse locomotive behaviors of animals are based, in part, on the developmental and evolutionary history of joint morphogenesis. We showed previously that strictly coordinated cell-differentiation and cell-movement events within the epidermis sculpt the interlocking ball-and-socket joints in the adult *Drosophila* tarsus (distal leg). Here, we show that the tarsal joints of various insect species can be classified into three types: ball-and-socket, side-by-side and uniform. The last two probably result from joint formation without the cell-differentiation step, the cell-movement step, or both. Similar morphological variations were observed in *Drosophila* legs when *Notch* function was temporarily blocked during joint formation, implying that the independent acquisition of cell differentiation and cell movement underlay the elaboration of tarsal joint morphologies during insect evolution. These results provide a framework for understanding how the seemingly complex morphology of the interlocking joint could have developed during evolution by the addition of simple developmental modules: cell differentiation and cell movement.

KEY WORDS: Joint morphology, Notch signaling, Cuticle, Evolution, Insect leg, *Drosophila*

INTRODUCTION

The motility of a joint depends largely on its morphology. The joints within individual animals display diverse morphologies (ball-and-socket, hinge, plane, etc.) and homologous joints in different species often have distinct morphologies (e.g. Romer and Parsons, 1977). The process of joint morphogenesis during development and its evolutionary modifications constitute the basis for the diverse locomotive behaviors of animals.

The ball-and-socket joint is an elaborate structure allowing both the rigid connection and flexible movement of neighboring elements. We previously proposed a two-process model, in which the conjunction of cell differentiation and cell movement during development sculpts the joint morphology of the adult *Drosophila* tarsus (distal leg) (Tajiri et al., 2010) (Fig. 1A). First, in the cell-differentiation step, cells in the future joint region invaginate to form a cavity during early pupation. The cells within the cavity resolve into two cell populations that commit to ball-producing or socket-producing cell fates and begin to produce cuticle that differs in shape and ultrastructure. In the cell-movement phase, which happens during cuticle secretion, both cell populations move their apical surfaces extensively, such that socket-producing cells wrap around the ball cuticle. The two processes must be tightly coordinated to form the tightly interlocking joint structure.

Here, we performed a comparative morphological analysis of insect species and other arthropoda and found that the two processes probably arose independently during insect evolution. In *Drosophila*, Notch activity controls both cell differentiation and cell movement through separate pathways, which might account for the modular nature of the two processes in joint development and evolution.

MATERIALS AND METHODS

Drosophila melanogaster strains

Strains used were UAS-GFP-TTras (Kato et al., 2004), *big brain*^{NP5149} (*bib*-GAL4) (Hayashi et al., 2002), *neuralized*^{P72} (*neur*-GAL4) (Bellaiche et al., 2001), *Notch-lacZ* (de Celis et al., 1998), Notch Response Element-*lacZ* (NRE-*lacZ*) (Furriols and Bray, 2001), N^{ts1} (FBst0002533), FM7i (FBba0000226), UAS-Dl::NΔECN (Nact) (FBst0005830), *ptc*-GAL4$^{559.1}$ (FBal0138169), UAS-mCD8::GFP (FBst0005137), tubP-GAL80^{ts} (FBst0007019) (Bloomington Stock Center), and UAS-*Notch* RNAi (v1112, v27228 and v27229; Vienna *Drosophila* RNAi Center). For N^{ts} experiments, N^{ts1}/FM7i females were crossed with either *bib*-GAL4; UAS-GFP-TTras or *ptc*-GAL4, UAS-mCD8::GFP males. Male progeny (N^{ts1}/Y and control FM7i/Y, distinguished by FM7i's *Bar* eye phenotype) were incubated at 18°C and shifted to 32°C at different stages. The timing of the shift [hours after puparium formation (APF) spent at 18°C] was: Fig. 2A-E, indicated in Fig. 2F; Fig. 2G, 49 hours; Fig. 2H, 69 hours; Fig. 2I, 75 hours; Fig. 2J-L′, 55-56 hours; Fig. 3B,C, 47.5-55 hours. For the Nact and *N* RNAi experiments, *bib*-GAL4, tubP-GAL80^{ts}, or *neur*-GAL4, tubP-GAL80^{ts} flies were crossed with UAS-Nact or UAS-*N* RNAi flies. Progeny were incubated at 18°C for 29 hours (Nact) or 46-48 hours (*N* RNAi) APF and shifted to 28°C (Nact) or 32°C (*N* RNAi).

Arthropod cuticle preparations

The adult legs of the following species were stored in 70% ethanol, sectioned with a razor blade if necessary, dehydrated in ethanol and isopropanol, and mounted in Canada balsam (Roberts, 1986): *Drosophila melanogaster* (fruit fly, Diptera), *Tapinoma* sp. (ant, Hymenoptera), *Cyclommatus metallifer* (stag

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beetle, Coleoptera), *Tribolium castaneum* (flour beetle, Coleoptera), *Eretes sticticus* (water beetle, Coleoptera), *Acanthocoris sordidus*, *Stictopleurus* sp. (both stink bugs, Hemiptera), *Lethocerus indicus* (giant water bug, Hemiptera), *Appasus japonicus* (ferocious water bug, Hemiptera), *Acyrthosiphon pisum* (aphid, Hemiptera), genus unknown (thrip, Hemiptera), genus unknown (book lice, Psocoptera), *Hodotermopsis sjostedti* (termite, Isoptera), *Blatta lateralis* (cockroach, Blattaria), *Tenodera aridifolia* (mantid, Mantodea), *Neohirasea* sp. (stick bug, Phasmatodea), *Locusta migratoria* (grasshopper, Orthoptera), *Achetus domesticus* (cricket, Orthoptera), *Atachycines apicalis* (cave cricket, Orthoptera), *Orthetrum albistylum*, *Epiophlebia superstes* (both dragonflies, Odonata), *Ischnura senegalensis* (damselfly, Odonata), *Ephoron eophilum* (mayfly, Ephemeroptera), *Pedetontus unimaculatus* (bristletail, Archeognatha), *Thermobia domestica* (firebrat, Thysanura), *Armadillidium vulgare* (pillbug, Isopoda, Crustacea), genus unknown (centipede, Scutigeromorpha, Myriapoda) and *Hasarius* sp. (spider, Arachnida, Chelicerata).

Immunohistochemistry and microscopy

Immunohistochemistry and confocal and electron microscopy were carried out as described (Tajiri et al., 2010). For electron microscopy of the mayfly tarsal joints, for which only samples stored in 70% ethanol were available, the initial fixation was modified as follows: samples were directly fixed in 2.5% glutaraldehyde, 2% formaldehyde, 70% ethanol for 2 hours at room temperature, then in 2.5% glutaraldehyde, 2% formaldehyde, 0.1 M cacodylate buffer for 1 hour at room temperature, and then stored in the solution at 4°C. The subsequent treatments, starting with agarose embedding, were carried out as described. The following antibodies were used at the indicated dilution: chick anti-β-galactosidase antibody (Abcam, 1:100), mouse anti-β-galactosidase (Promega, 1:100), anti-Dl [C594.9B, Developmental Studies Hybridoma Bank (DSHB), 1:100] and anti-N (C17.9C6, DSHB, 1:50).

RESULTS AND DISCUSSION

Evolution of tarsal joint morphologies

To examine how the combination of cell differentiation and cell movement evolved to create the ball-and-socket tarsal joint, we compared the tarsal joint morphologies of various insect species. The ball-and-socket morphology was not found in the tarsal joints of the primitive insects Apterygota and Paleoptera. In the distal tarsal joint of the bristletail (Apterygota, Fig. 1D) and in all the tarsal joints of the mayfly (Paleoptera, Fig. 1E), the joint cavity was covered by a uniform, continuous cuticle. Electron microscopy revealed no ball-socket distinction within the cuticle of the mayfly joint (see Fig. S2 in the supplementary material). These findings suggested that all the cells in the invaginated region produced a single type of cuticle during tarsal joint development, instead of differentiating into two distinct (ball-producing and socket-producing) populations (Fig. 1A′c).

The proximal tarsal joint of the bristletail (Fig. 1C), the tarsal joints of the firebrat (Apterygota, see Fig. S1T,U in the supplementary material) and those of the damselfly and dragonfly (Paleoptera, Fig. 1F; see Fig. S1R,S in the supplementary material) consisted of two pieces of hard cuticle that lined the cavity and were positioned side by side, without one enwrapping the other. As the enwrapment of the ball by the socket in the *Drosophila* tarsal joint is achieved by cell movement during cuticle secretion, this side-by-side morphology is likely to represent joint formation without cell movement (Fig. 1A′b).

All three types of tarsal joints (ball-and-socket, side-by-side and uniform) were found in the Polyneoptera (see Fig. S1I-Q in the supplementary material). For example, the cave cricket contains all three types within a single tarsus (Fig. 1G-I). In the Paraneoptera and Holometabola, the ball-and-socket type was often found, for example in the stink bug (Hemiptera, Fig. 1J), stag beetle (Coleoptera, Fig. 1K) and ant (Hymenoptera, Fig. 1L), with some exceptions (see Fig. S1A-H in the supplementary material).

Given our findings, we propose that multiple, separate gains and losses of the two essential processes for ball-and-socket joint morphogenesis, (1) differentiation of ball-producing cells versus socket-producing cells and (2) cell movement, underlie the evolution of tarsal joint morphologies. The two processes may be considered to be ‘building blocks’ or ‘modules’ for different joint structures.

We found the ball-and-socket type in the Neoptera but not in relatively primitive insect species or other arthropod subphyla (Myriapoda, Crustacea and Chelicerata) (Fig. 1; see Fig. S1V-X in the supplementary material). Tarsal joints of this type might have evolved in the Neoptera through the acquisition of cell movement during cuticle secretion. Among the ball-and-socket joints, the ‘ball’ morphology varies substantially, ranging from literally ball-like globular shapes in *Drosophila* (Fig. 2) and the ant (Fig. 1L) to thin, stick-like ones in the stink bug (Fig. 1J) and the book lice (see Fig. S1D in the supplementary material). Even in the side-by-side joints, the morphology of the distal cuticle varied, from a rod shape in the damselfly (Fig. 1F) to a branched structure in the distal-most joint of the cave cricket (Fig. 1I). The ball-and-socket and side-by-side joint types might have evolved more than once, giving rise to these distinct structures. Alternatively, the ball morphology might have been differentially modified in different lineages; for example, it could have undergone additional swelling in *Drosophila* and the ant, or thinning in the stinkbug. If so, the regulation of ball morphology might be another module of joint morphology evolution, acting in parallel with cell differentiation and cell movement.

An important evolutionary question is how the different morphologies of tarsal joints affect organism fitness. As the number of tarsomeres appears to be uncorrelated with the class(es) of tarsal joint morphologies in each species (Fig. 1B), it is difficult to assess how the flexibility of individual joints contributes to the mobility of the tarsus. We suspect that tarsal joint morphology has minimal impact on whole-leg motions, such as walking and jumping, because larger, muscle-containing segments, including the tibia and the femur, are more likely to effect these motions.

Intriguingly, the ventral surfaces of tarsomeres in many insects have adhesive pads and sensilla (Chapman, 1998). The ball-and-socket joint, which is presumably more flexible than the other types, might permit more tarsomeres of a single limb to fit to curved or jagged surfaces, allowing better substrate attachment and sensing. However, the uniform and side-by-side joint types might have different advantages. The appearance of side-by-side joints in some Holometabolous insects and the co-existence of different types within a single tarsus in some Polyneoptera suggest that tarsal joint morphology has not evolved in a linear fashion from uniform to side-by-side to ball-and-socket. Rather, different ecology- and physiology-dependent selective pressures seem to have resulted in the evolution of any or all three joint types in individual species.

Knockdown of Notch function during pupal development in *Drosophila* converts its interlocking joint to a more primitive type

The Notch signaling pathway has a central role in the segmentation of the arthropod leg. In *Drosophila melanogaster*, loss of Notch function that begins in the larval period impairs the initial invagination, and blocks the development of joint structure (Bishop et al., 1999; de Celis et al., 1998; Rauskolb and Irvine, 1999). In an attempt to ‘freeze’ joint morphogenesis at different phases, we knocked down Notch function at different times during pupa, using a temperature-sensitive allele of *Notch* (N^{ts}). Mutants were reared

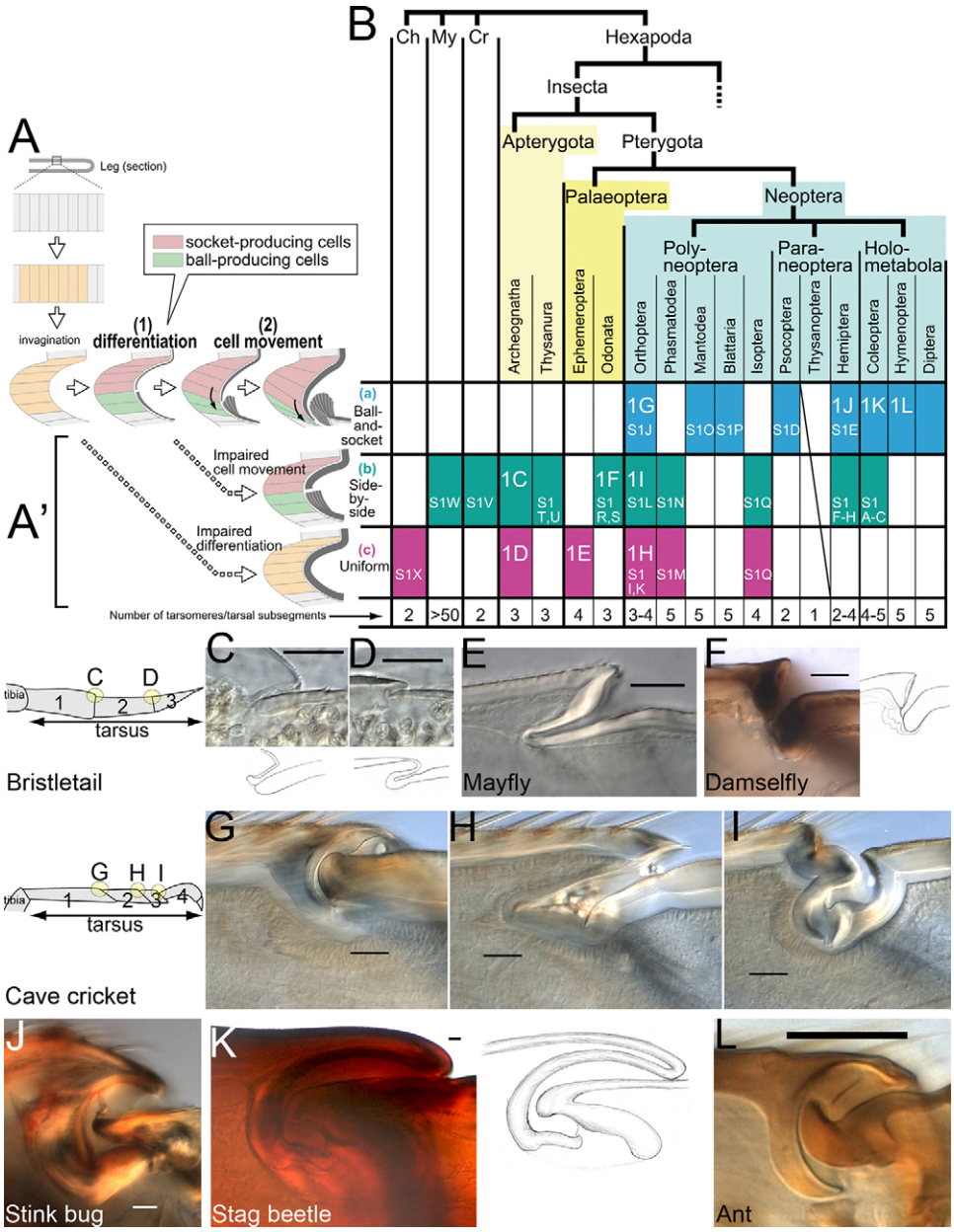

Fig. 1. Tarsal joint morphologies. (**A**) Normal morphogenesis of *Drosophila* tarsal joint. (**A′**) Presumptive joint morphology that would result from impaired cell movement (b, side-by-side) or from impaired differentiation of ball-producing versus socket-producing cells (c, uniform cuticle). (**B**) Tarsal joint morphologies in various insect orders and other arthropod subphyla. Blue, green and red in the table indicate the presence of ball-and-socket, side-by-side and uniform tarsal joints, respectively. Figure numbers of representative images are indicated in white. Numbers at the bottom indicates the number of tarsomeres in the examined species. (**C-L**) Tarsal joints of various insect species. Scale bars: 10 μm. (C,D) Bristletail joints between tarsomeres 1 and 2 (C, side-by-side), and between tarsomeres 2 and 3 (D, uniform). Cuticle morphologies are represented by drawings in lower panels. (E) Mayfly (uniform). (F) Damselfly (side-by-side). (G-I) Cave cricket, joints between tarsomeres 1 and 2 (G, ball-and-socket), 2 and 3 (H, uniform), and 3 and 4 (I, side-by-side). (J) Stink bug (ball-and-socket). (K) Stag beetle (ball-and-socket; drawing on the right). (L) Ant (ball-and-socket). In all images, dorsal is up and distal is to the right.

at 18°C (permissive temperature), shifted to 32°C (restrictive) at different times during the pupal period and maintained at 32°C until adulthood.

Mutants raised at 18°C throughout development or shifted to 32°C after the onset of cuticle secretion had normal tarsal joints (Fig. 2E,F), and a shift to 32°C during the prepupal period sometimes blocked invagination (Fig. 2A,F), consistent with Notch's known essential role in inducing invagination. Shifts at intermediate stages resulted in a range of joint phenotypes (Fig. 2F), as follows.

Flies shifted around or shortly after pupation showed a uniform, continuous cuticle that covered the entire joint cavity (Fig. 2B), which was strikingly reminiscent of the 'primitive' tarsal joints seen in the mayfly (Fig. 1E). Electron microscopy confirmed the uniform nature of this cuticle (Fig. 2G,G′). Notably, none of the uniform cuticle showed distinct layers, which is a hallmark of the ball cuticle (Tajiri et al., 2010) (layers are visible in Fig. 2I and Fig. 3H″). Additionally, the lubricant was severely reduced. In some cases, the uniform cuticle had a projection on the ventral side (Fig. 2C,H). Later shifts resulted in an almost-normal ball-and-socket morphology, except that the ball and socket abutted each other instead of being separated by lubricant (Fig. 2D,I, arrows in I′).

These observations indicated that, in *Drosophila*, Notch function during the early pupal period is required for the correct ball-socket distinction in the cuticle morphology and ultrastructure.

Cell movement is separable from the ball-socket distinction

In normal *Drosophila* joint morphogenesis, elongation of the ball 'lip' (Fig. 2E) and the socket coincides with the movement of the cells secreting the components that form them (Tajiri et al., 2010). We hypothesized that the occasional lip-like projection on the uniform cuticle in N^{ts} mutants (Fig. 2C,H) represented cell movement during cuticle secretion. We labeled a subset of cells with a membrane-tethered GFP and examined whether their movement correlated with cuticle morphology. In controls, the

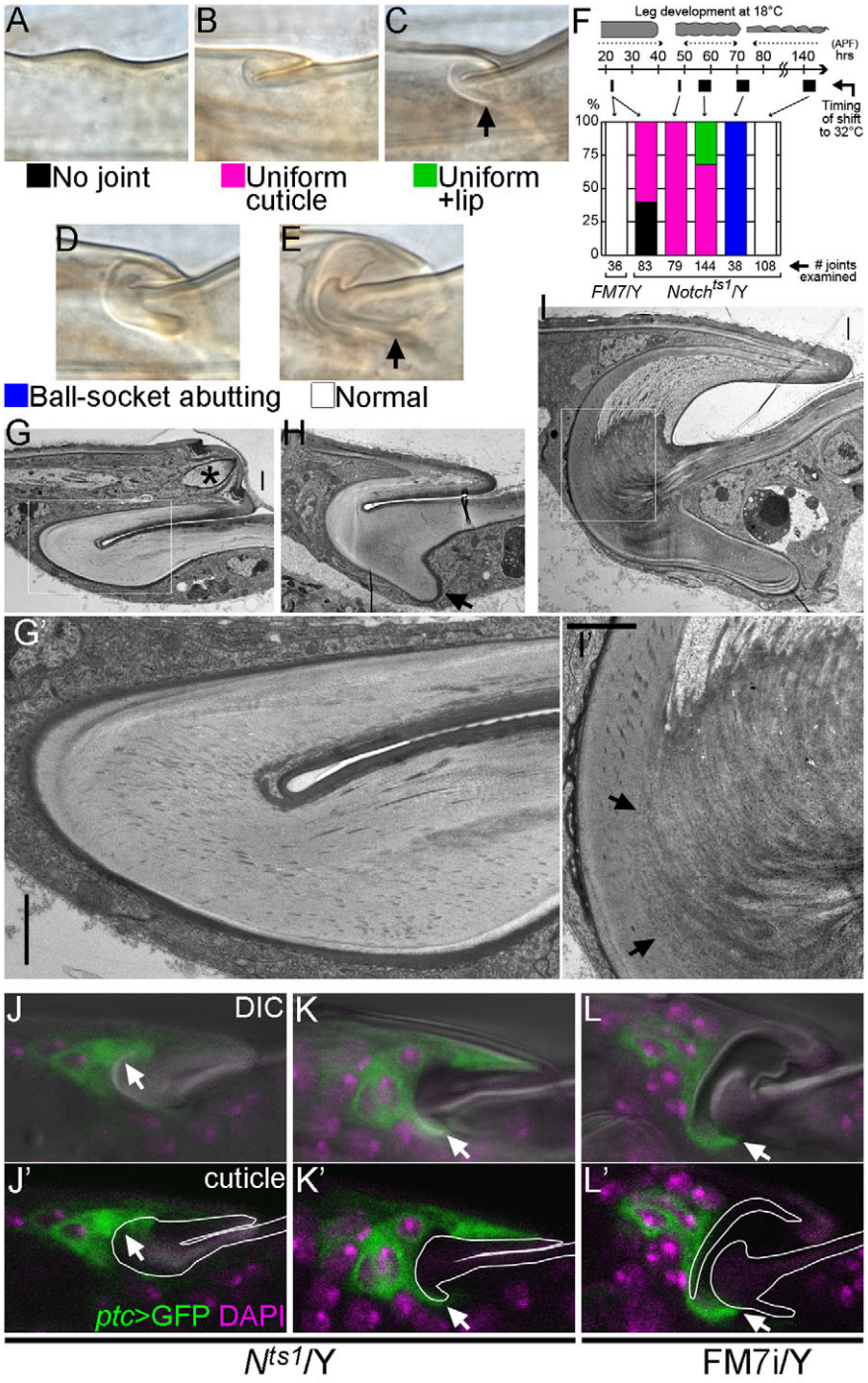

Fig. 2. Tarsal joint morphologies in *Drosophila N^ts^* mutant. (**A-F**) Phenotypic classes of tarsal joint morphologies in N^{ts1} mutants raised at 18°C and shifted to 32°C at different stages during the pupal period. Phenotypes ranged from no joint (no invagination, A, indicated in black in F), uniform cuticle covering the cavity (B, magenta in F), uniform cuticle with a ventral projection ('lip') (C, arrow indicates projection, green in F), no spacing between the ball and socket (D, blue in F), to the normal ball-and-socket morphology (E, arrow indicates ball lip, white in F). Sibling FM7*i*/Y ('FM7/Y') flies served as controls. In F, hours after puparium formation (APF) at 18°C is shown along the leg morphologies at different stages. Horizontal black bars indicate when temperature shifts were conducted. The frequencies of phenotypes caused by each shift are shown below. (**G-I'**) Transmission electron micrographs of adult N^{ts} tarsal joints. (G) 'Uniform cuticle' (boxed region magnified in G'). This phenotype was sometimes associated with ectopic cuticle accumulation outside the joint invagination (asterisk). (H) 'Uniform cuticle with a lip.' Arrow indicates lip. (I) 'Ball-socket abutting' (boxed region shown in I'). Arrows indicate ball-socket interface. Scale bars: 1 μm. (**J-L'**) Cell movement in N^{ts1} mutant. Cells expressing mCD8::GFP driven by *ptc*-GAL4 in N^{ts1}/Y (J-K') and control FM7*i*/Y (L,L') were observed when cuticle formation was close to completion. Cuticles visualized by differential interference contrast (DIC) in the upper panels were manually traced in white in the lower panels. Arrows indicate the apical surfaces of labeled cells, which had moved along with the cuticles (K-L') or remained in their original position (J,J'). In all images, dorsal is up and distal is to the right.

apices of the labeled cells continuously contacted the ends of the ball-and-socket cuticles, moving from the original dorsal position to the final ventral position (Fig. 2L,L′) (Tajiri et al., 2010). In mutant uniform joints with a projection, the labeled cells likewise moved their apical surfaces along the cuticular projection (Fig. 2K,K′). In uniform joints without a projection, the apical surfaces of the labeled cells remained in the dorsal-proximal region (Fig. 2J,J′).

These results show that Notch activity in the early pupal period is required for cell motility. The occurrence of cell movement regardless of impairment of the ball-socket distinction indicates that the two processes can be uncoupled. They are both controlled by Notch signaling, but probably through independent pathways. Taken together with our previous finding that cell movement proceeds even when the cuticle structure is severely disrupted (Tajiri et al., 2010), we propose that Notch signaling activates a cell-intrinsic mechanism that drives the movement of cell apical surfaces. The uncoupling of the ball-socket distinction and cell movement supports our hypothesis that the two steps have served as evolutionary 'modules' to permit variation in joint structures.

Difference in Notch activity levels promotes the ball-socket distinction

Ball-producing cells express *big brain* (*bib*) (Tajiri et al., 2010), a positive readout of Notch signaling activity (de Celis et al., 1998). Consistent with this, *bib* expression coincided with the strong

expression of Notch Response Element-*lacZ* (NRE-*lacZ*) (Furriols and Bray, 2001) (Fig. 3A), and was significantly diminished in the N^{ts} mutant at 32°C (Fig. 3B,C). As mentioned above, the absence of ball-like characteristics in the uniform cuticle of N^{ts} (Fig. 2G,G′) indicated that the ball-producing activity was compromised. We therefore hypothesized that Notch activity contributes to the distinction between the ball and the socket by promoting ball production. To test this, we manipulated Notch signaling activity in a cell-specific manner and examined the effects on ball/socket formation.

When a constitutively active form of Notch (Nact) was expressed using *bib*-GAL4 (in the ball-producing cells and neighboring socket-producing cells), the socket cuticle was considerably shorter than normal, but the ball cuticle retained its normal morphology (Fig. 3D). Expression of Nact with *neur*-GAL4 (only in socket-producing cells) resulted in a similar phenotype (Fig. 3E). Electron micrographs confirmed the absence of the socket cuticle in the lateral and ventral regions (Fig. 3H-H″). Thus, excess Notch activity within the cells that would normally produce the socket interferes with socket production, but it does not cause any significant abnormality in the ball-producing cells.

Expression of an RNAi against *Notch* driven by *bib*-GAL4 caused a phenotype resembling the uniform cuticle of the N^{ts} mutant (Fig. 3F). A small portion of the cuticle exhibited a layered organization in electron micrographs (Fig. 3I), which we assume was trace ball cuticle. In some cases, a small, incomplete ball

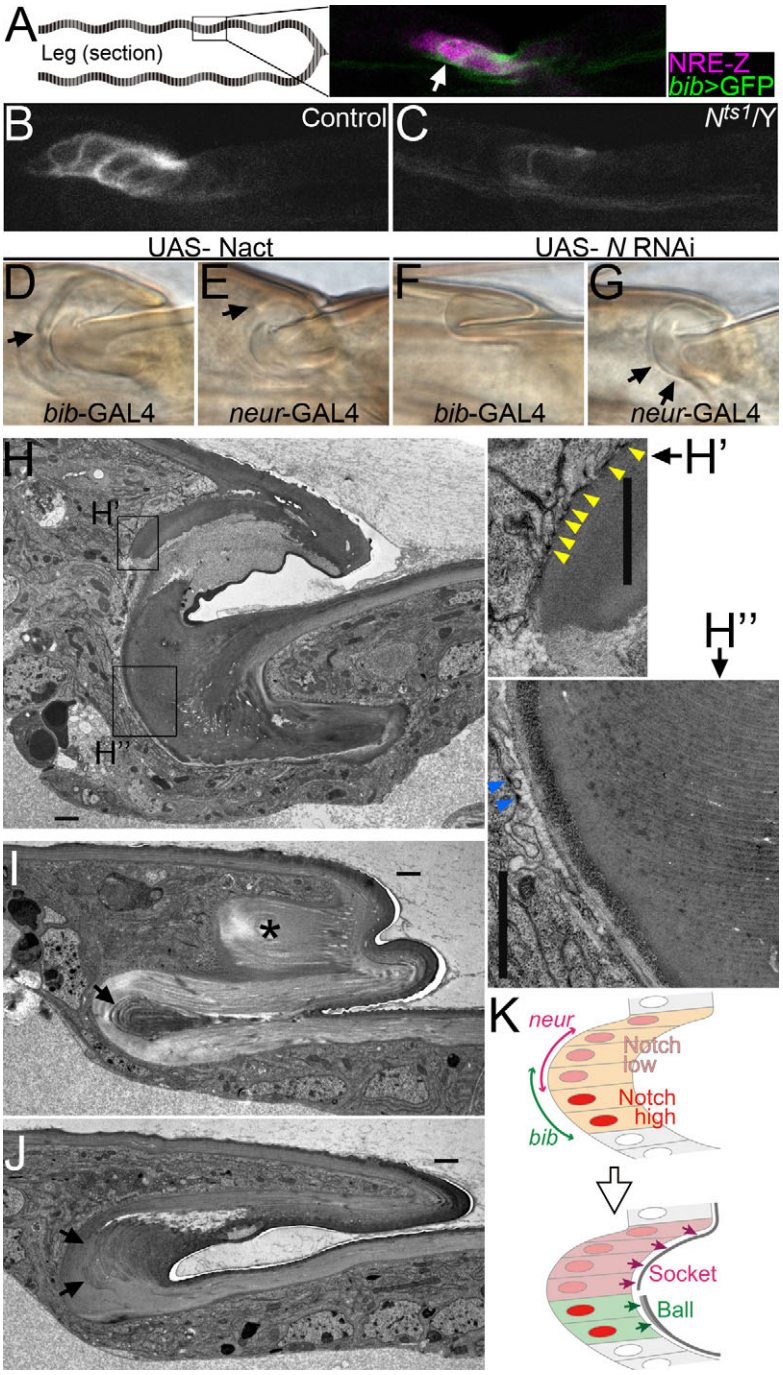

Fig. 3. Differential Notch activities promote the ball-socket distinction. (**A**) Expression of NRE-*lacZ* and *bib*-GAL4 in the joint region, examined at the onset of invagination. Strong expression of NRE-*lacZ* (magenta) coincides with that of *bib*-GAL4 (green; arrow). (**B**,**C**) Expression of membrane-bound GFP (GFP-TTras) controlled by *bib*-GAL4 in FM7*i*/Y (B) and N^{ts1}/Y (C). Images were taken under the same microscopy conditions. (**D-G**) Tarsal joints in which Nact (D,E) or dsRNA against *Notch* (F,G) were expressed using *bib*-GAL4 (D,F) or *neur*-GAL4 (E,G). Arrows indicate the end of the socket cuticle (D,E) or thin socket (G). (**H-H″**) Electron micrograph of a tarsal joint in which Nact was driven by *neur*-GAL4. Boxed regions are magnified in H′ and H″. The socket ends dorsally (H′) and does not extend to the lateral and ventral sides of the ball (H″). Socket-producing cells contact the socket cuticle via plasma membrane plaques (PMPs, yellow arrowheads in H′), but the presumptive *neur*-expressing cells do not contact the ball cuticle via PMPs (H″, blue arrowheads indicate PMPs that do not contact the ball). (**I**,**J**) Electron micrographs of tarsal joints in which dsRNA against *Notch* was expressed under *bib*-GAL4. In I, a small portion of cuticle exhibits a layered organization (arrow). Asterisk indicates cuticle accumulation outside the joint invagination, which is also observed in N^{ts} (Fig. 2G). In J, a small, incomplete ball abuts the socket (arrows indicate the interface). Scale bars: 1 μm. (**K**) Model of the ball-socket distinction by differential levels of Notch activity. High Notch-signaling levels (red nuclei) promote ball production, whereas low levels (pink nuclei) are required for socket production. The expression domains of *bib* and *neur* are indicated by arrows. In all images, dorsal is up and distal is to the right.

cuticle formed and fused with the socket (Fig. 3J). These observations suggested that ball production was significantly reduced, as in the N^{ts} mutant. When the RNAi was driven by *neur*-GAL4, the socket cuticle was thinner than normal (Fig. 3G), indicating that a certain level of Notch signaling is required in the socket-producing cells.

These results are compatible with the model shown in Fig. 3K: high levels of Notch signaling promote ball production, and lower levels are required for socket production. It is presently unclear at which phase Notch signaling regulates these activities. It might be during the fate specification of ball-producing versus socket-producing cells, and/or during their differentiation and maintenance, in which Notch might direct the expression of specialized cuticular components and regulators of cell movement, etc. We note that the expression of Nact in *neur*-expressing cells did not convert them into ball-producing cells; electron micrographs showed that the presumptive *neur*-expressing cells (judged by their location) did not contact the ball cuticle through the plasma membrane plaques, as ball-producing cells would do (Fig. 3H″) (Tajiri et al., 2010). Thus, Notch signaling seems to act in more ways than as a simple binary cell-fate switch.

We examined the expression of Delta (Dl), a Notch ligand, and of Notch itself during and shortly after the onset of invagination (the sensitive period in the N^{ts} experiment). Anti-Notch immunostaining revealed a sharp boundary between the distal cells, with high Notch levels, and the proximal cells with low Notch levels; this pattern was corroborated by the expression of *Notch-lacZ* (see Fig. S3A in the supplementary material). Delta accumulated at high levels in the most proximal row of *Notch-lacZ*-expressing cells (see Fig. S3B,C in the supplementary material), which coincided with the proximal end of the *bib* expression domain (high Notch-signaling levels; see Fig. S3D in the supplementary material). Thus, Notch is activated in cells distal to the Dl-expressing cells (corresponding to future ball-producing cells), whereas the proximal cells (corresponding to socket-producing cells) have lower Notch-signaling levels.

This spatial relationship between ligand expression and Notch signaling activity is identical to that reported for leg discs at prepupal stages, when *fringe*, *frizzled* and *dishevelled* are proposed to repress Notch signaling in cells proximal to the ligand-expressing ones (Bishop et al., 1999). The same mechanism might maintain the differential Notch signaling in the distal versus proximal cells at the pupal stage. In addition, different levels of Notch expression itself (see Fig. S3A in the supplementary material), probably resulting from positive feedback (Bishop et al., 1999), might contribute to the differential signaling levels.

In conclusion, we have shown that, in *Drosophila*, the two essential processes for ball-and-socket joint morphogenesis, differentiation of ball-producing cells versus socket-producing cells and cell movement, are regulated by Notch signaling through separate pathways. The variety of tarsal joint morphologies among insects is likely to have been generated by independent evolutionary acquisition and/or loss of these processes. These results provide a framework for clarifying the cellular and molecular mechanisms underlying the developmental regulation of joint morphology, and for understanding how those mechanisms have served evolutionarily as building blocks for their variations. In future studies it will be important to investigate how, and to what extent, the levels and downstream cascades of Notch signaling have been modulated in the individual tarsal joints of other insects, and to determine how this modulation is linked to the evolution of joint morphologies.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material for this article is available at
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