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Why should transverse rows need the EGFR pathway to align properly on *Drosophila* legs?

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Most bristles on the legs of a fruit fly are organized in longitudinal ("l") rows that run parallel to the proximal-distal axis. However, the forelegs and hindlegs also have ventral areas where bristles form rows that are perpendicular to l-rows (Hannah-Alava, 1958). These transverse ("t") rows look remarkably like hairbrushes and are used as such during the cleaning ritual (Szebenyi, 1969). The foreleg's t-rows reside anteriorly and clean the eyes, while the hindleg's t-rows reside posteriorly and clean the wings. The t-rows adorn the basitarsus and distal tibia. The distalmost t-row on the male basitarsus becomes the "sex comb", whose bristles are dark, thick, and blunt (Tokunaga, 1962).

The t-rows are intriguing because of their special features relative to l-rows. Unlike the bristles in each l-row, which are separated by intervals equal to several socket diameters, the bristles in each t-row lack intervening spaces. That is, their sockets touch (Figure 1a). How such tandem arrays arise is not known (Held, 1991).

During an investigation of bract induction (Held, 2002), I discovered some odd phenotypes that may offer clues about t-row patterning. By manipulating the Epidermal Growth Factor Receptor (EGFR) pathway, the t-rows can be altered. For example, t-rows become disorganized when the EGF receptor (Egfr) is overexpressed via *scabrous-Gal4*, or when an activated Ras1 is expressed for 1 hour via a heat-shock promoter at any time through 22 hours after pupariation at 25˚C (data not shown; see Held, 2002). Chaos of this sort is also seen when the Notch pathway is disabled (see Held, 1990), but in that case the number of bristles also changes (increases), which complicates the analysis.

To dissect the temporal requirement for Egfr, I used a temperature-sensitive allele ($Egfr^{ts1a}$; Kumar *et al*., 1998) and a null allele ($Egfr^{CO}$; Clifford and Schüpbach, 1989). $Egfr^{ts1a}/Egfr^{CO}$ heterozygotes were raised at 18˚C (permissive temperature) throughout the larval period to avoid lethality. In the "upshift" protocol, cohorts were shifted to 29˚C (restrictive temperature) at various times after pupariation. In the "downshift" protocol, cohorts were shifted to 29˚C at pupariation and returned to 18˚C at various times. In both series, successive cohorts were shifted at 2-hour intervals over a 36-hour span, and 6 male forelegs were examined per time point. Pupal ages are reported as "hAP25" = hours after puparation normalized to the standard 25˚C pace of development. Conversion factors were 0.5× for 18-to-25˚C and 1.16× for 29-to-25˚C (Held, 2002). Legs were mounted in Faure's solution between cover slips and examined at 200× magnification.

Upshifts between 0 and 10 hAP25 produce recognizable t-rows (Figure 1b), but bristles within each row are jumbled in irregular groups, instead of forming a single file, and rows are often merged. The distalmost row remains relatively normal. Upshifts after 10 hAP25 have little effect—implying that EGFR signaling (disabled by upshift) is no longer needed after this time.

Downshifts between 0 and 14 hAP25 have little effect—implying that the need for EGFR signaling can be fulfilled at any time during this window. Downshifts after 21 hAP25 yield

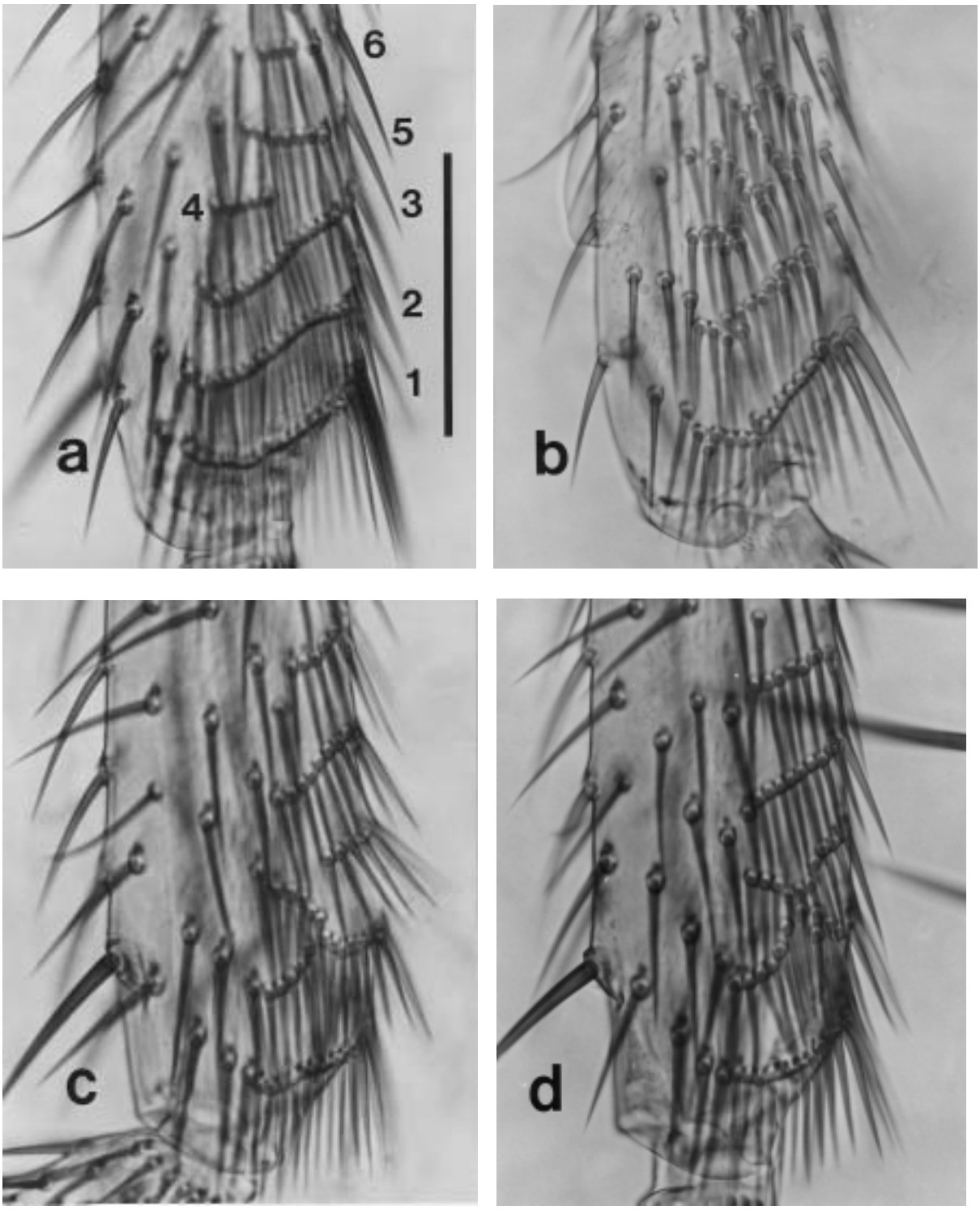
phenotypes (not shown) that are essentially like those from upshifts at 0-10 hAP25—implying that turning on the EGFR pathway after this time is too late to rescue any alignment function.

Novel phenotypes were seen with downshifts between 14 and 21 hAP25. Most legs from these cohorts (87%) are normal, but in a minority of cases (13%) adjacent rows bend toward one another and join at Y- or X-shaped intersections (Figures 1c-d). Evidently, activating the pathway at this stage prevents clumping within t-rows but cannot reliably prevent connections between adjacent t-rows.

In summary, tibial t-row bristle cells need the EGFR pathway to align properly. They normally finish using the pathway before 10 hAP25, but they can still use it until 21 hAP25—the end of their competence to be rescued (in accord with the Ras1-sensitive period, which lasts until 22 hAP25). The pathway is apparently needed for two sequential alignment steps: (1) the separation of rows from one another into parallel files and (2) the alignment of bristles within each row into a single file.

Scale cells in moth wings coalesce into chains by moving within the epidermis (Nardi and Magee-Adams, 1986). Assuming that bristle cells do the same on fly legs, what remains unclear is why they would use a pathway that relies on *diffusible* signals (*cf*., Beccari *et al*., 2002; Fagotto and Gumbiner, 1996; Rebay, 2002), rather than *contact-mediated* signals (*cf*. Nardi, 1992, 1994). Might EGFR be playing a gradient role in the t-row area analogous to its recently revealed role in the tarsus (Campbell, 2002; Galindo *et al*., 2002)?

Figure 1. Transverse rows on the forelegs of male $Egfr^{ts1a}/Egfr^{CO}$ heterozygotes. Each panel shows the anterior face of the distal end of a right-leg tibia, with distal at the bottom, dorsal to the left, and ventral to the right. The scale bar in panel "a" is 100 microns long. (a) Tibia of a fly raised at 18˚C. The t-row pattern looks wild-type. There are 6 t-rows, numbered from distal to proximal. The 4th row is only partial, and the neighboring rows (3 and 5) bend around it so as to maintain a constant inter-row interval. Partial rows are a normal (albeit infrequent and usually L/R asymmetric) feature of wild-type flies. Note that within each row the bristle sockets touch in a single file. In l-rows (visible outside the triangular t-row area) bristles are separated by large intervals. (b) Tibia of a fly shifted from 18˚C to 29˚C at 18 hAP (≈ 9 hAP25). Although the distalmost t-row is fairly normal, the more proximal ones are disturbed, with irregular clumps of sockets and mergers of rows. (c) Tibia of a fly shifted from 29˚C to 18˚C at 12 hAP (≈ 14 hAP25). The t-rows look wild-type insofar as the sockets are in single file within each t-row, but the 2nd and 3rd rows intersect. No Y-shaped link like this has ever been observed among many hundreds of wild-type legs (L. Held, pers. obs.), though such "triradii" are common in human fingerprints (Cummins and Midlo, 1943). (d) Tibia of a fly shifted from 29˚C to 18˚C at 18 hAP (≈ 21 hAP25). Again, t-rows look normal except that 2nd and 3rd rows intersect—in this case, at a novel X-shaped juncture.



References: Beccari, S., L. Teixeira, and P. Rørth 2002, Mechs. Dev. 111: 115-123; Campbell, G., 2002, Nature 418: 781-785; Clifford, R.J., and T. Schüpbach 1989, Genetics 123: 771-787; Cummins, H., and C. Midlo 1943, *Finger Prints, Palms and Soles. An Introduction to Dermatoglyphics.* Dover, New York; Hannah-Alava, A., 1958, J. Morph. 103: 281-310; Held, L.I., Jr., 1990, Roux's Arch. Dev. Biol. 199: 48-62; Held, L.I., Jr., 1991, BioEssays 13: 633-640; Held, L.I., Jr., 2002, Mechs. Dev. 117: 225-234; Fagotto, F., and B.M. Gumbiner 1996, Dev. Biol. 180:

445-454; Galindo, M.I., S.A. Bishop, S. Greig, and J.P. Couso 2002, Science 297: 256-259; Kumar, J.P., M. Tio, F. Hsiung, S. Akopyan, L. Gabay, R. Seger, B.-Z. Shilo, and K. Moses 1998, Development 125: 3875-3885; Nardi, J.B., 1992, Dev. Biol. 152: 161-171; Nardi, J.B., 1994, Dev. Dynamics 199: 315-325; Nardi, J.B. and S.M. Magee-Adams 1986, Dev. Biol. 116: 265-277; Rebay, I., 2002, Dev. Biol. 251: 1-17; Szebenyi, A.L., 1969, Anim. Behav. 17: 641-651; Tokunaga, C., 1962, Dev. Biol. 4: 489-516.