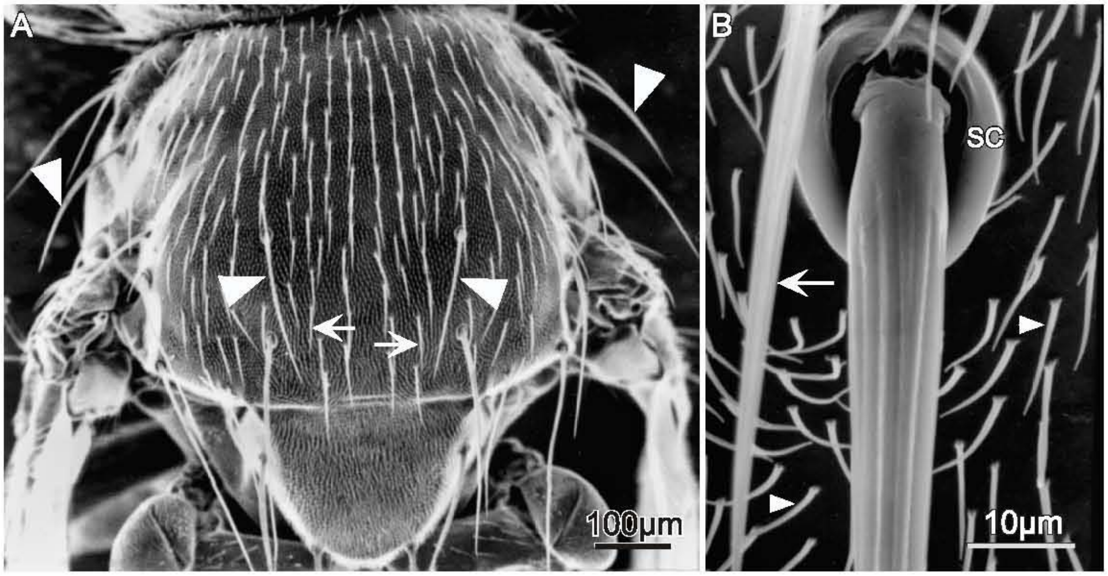

Figure s1

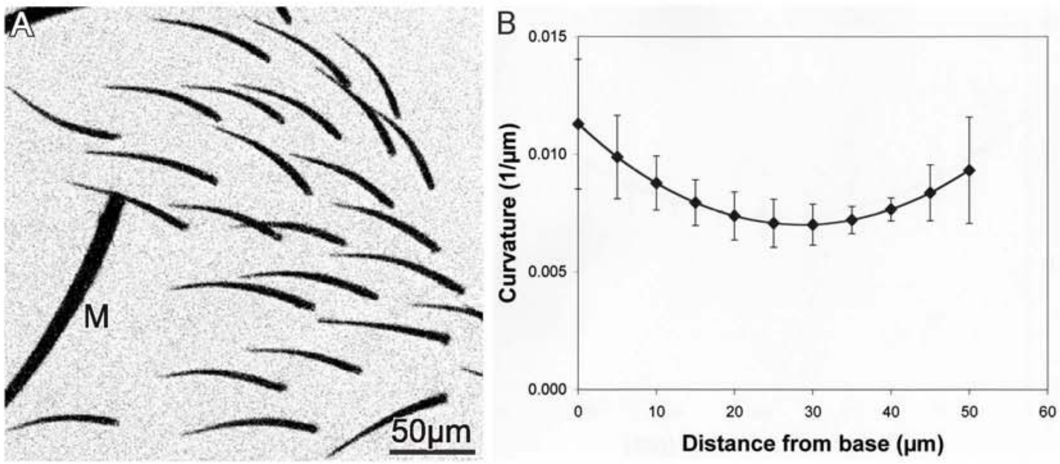
A
M
50μm
B
Curvature (1/μm)
0.015
0.010
0.005
0.000
0
10
20
30
40
50
60
Distance from base (μm)

Figure s2

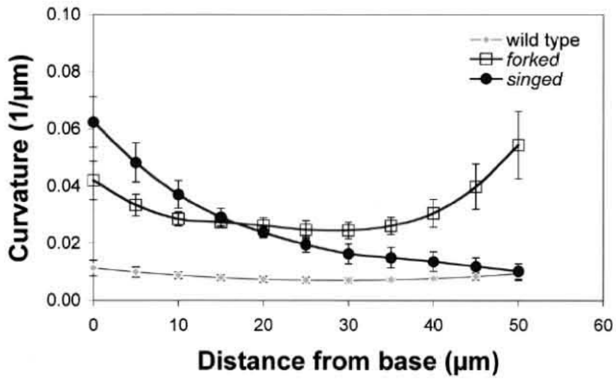
0.10
0.08
0.06
0.04
0.02
0.00
Curvature (1/µm)
wild type
forked
singed
0
10
20
30
40
50
60
Distance from base (µm)

Figure s3

Supplemental Material for Tilney et al.

Figure s1
Figure s2
Figure s3

Supplementary Figure Legends

Figure s1. Scanning electron micrographs of *Drosophila* bristles. A) Dorsal thorax. Of interest to this report are the bristles present on the dorsal thorax. The arrowheads point to four of the 22 macrochaetes present on the dorsal thorax. The 200 smaller bristles or microchaetes (arrows) all extend posteriorly over the thorax. Bar, 100 μm. B) The basal portion of a single microchaete at higher magnification. To the left is a tip of a second microchaete (arrow). The shafts of these bristles are fluted. Notice that the basal end of the microchaete extends from socket cell (SC). Small hairs or extensions of the epidermal cells surround the bristles (arrowheads). Bar, 10 μm.

Figure s2 The curvature of the wild-type microchaetes visualized by blotting. A) Image of a typical blot of wild-type microchaetes. The basal end of a larger macrochaete (M) is also present in this light micrograph. Bar, 50 μm. B) The curvature of wild-type microchaetes was measured and plotted as a function of bristle length. The greatest curvature is near he bristle base. Curvature decreases only to then increase again at the tip. Bars, SEM.

Figure s3 Curvature measurements of wild-type and mutant bristles. The curvature of wild-type, *forked* and *singed* microchaetes was measured from light micrographs of blots and plotted as a function of bristle length. Bars indicate the standard error of the mean.

Can actin bundle treadmilling generate bristle curvature? As mentioned in the text, this mechanism predicts that module length in the actin bundles will be inversely proportional to the radius of curvature and not proportional to the square of radius of curvature. To check to see if this possibility fits the existing data we wrote the following equations using the variables indicated below.

Variables:

- L=initial length of the bundle
- _L=extension during grafting of segments
- R=radius of curvature of the bristle
- _=the angle such that R_=L+_L
- a=the number of actin subunits
- r=radius of the bundle
- D=bundle separation (≈bristle diameter)
- s=superior subscript
- i=inferior subscript

N=number of filaments in the bundle
n=number of filaments on periphery of bundle
t=time
k=delivery constant subunits/(second*filament)
_=filament separation

The number of actin filaments at the periphery of the bundle is:

$$n = 2\pi r / \delta$$

The number of actin subunits delivered to the end of the bundle is:

$$a = nkt = 2\pi rkt / \delta$$

The number of filaments in the bundle is:

$$N = \pi r^2 / \delta^2$$

The increase in length is equal to the number of filaments (that can accommodate the motors) times the rate of delivery of actin subunits per unit time times the time times the length increment per actin subunit added:

$$\Delta L(nm) = 2.7a / N = \frac{2 \bullet 2.7\pi rkt}{\delta} \frac{\delta^2}{\pi r^2} = \frac{5.4\delta kt}{r}$$

The length of the bundle is related to the curvature and the angle:

$$R\alpha = L + \Delta L_S = L + \frac{5.4\delta kt}{r_S}$$

The different lengths of the superior and inferior elongated bundles determine the angle

$$\alpha = \frac{\Delta L_S - \Delta L_I}{d} = \frac{\left(\frac{1}{r_S} - \frac{1}{r_I}\right)5.4\delta kt}{d}$$

Substituting in:

$$\frac{R\left(\frac{1}{r_S} - \frac{1}{r_I}\right)5.4\delta kt}{d} = L + \frac{5.4\delta kt}{r_S}$$

The curvature (1/R) is obtained by rearranging terms:

$$\frac{\left(\frac{1}{r_S}-\frac{1}{r_I}\right)5.4\delta kt}{d\left(L+\frac{5.4\delta kt}{r_S}\right)}=\frac{1}{R}$$

Rearranging terms again:

$$\frac{1}{R}=\frac{(r_I-r_S)}{dr_I\left(\frac{Lr_S}{5.4\delta kt}+1\right)}$$

The idea is to use the measurable curvature, bundle radii, and distance (d) at the bristle base to determine L/(5.4_kt) and then apply it to other points along the bristle. This of course assumes that the determined L/(5.4_kt) is constant with distance along the bristle.

We decided to determine the value for L/(5.4kt) for various sections on the grounds that it should be approximately constant. The formula used was

$$\frac{L}{5.4kt}=\frac{\delta}{r_S}\left(\frac{r_I-r_S}{dr_I\frac{1}{R}}-1\right)$$

When we did this on data (Table 1) included in Tilney et al. (2000a) the values ranged from 0.9 to 9.4 with one anomalous value of 28.4. The mean was 6.5 with a standard deviation of 8.1 (which includes the odd value) (n=10). Without the odd value, the mean and standard deviation are 4.1 and 2.6 respectively (n=9).

We then figured out what the predicted length _L assuming that L=1000 nm:

$$\frac{L}{5.4kt}\approx 4$$

$$5.4kt=L/4\approx\frac{1000}{5}=250$$

$$\Delta L=\frac{5.4kt\delta}{r}\approx\frac{250*11}{100}\approx 30$$

This would mean that the average increase in length of the modules is 30nm, which is much too short. If we assume that initial module length is approximately 1 μm (Fig 3) and that the average module length in microchaetes of 3.1 μm (Tilney et al., 1996), we expect that module length would increase at least three-fold from 1000 nm to 3000 nm. Also there is a trend in the L/(5.4kt) that is correlated with the diameter of the cross section, which presumably determines its position along the bristle. Thus, the equations used show that treadmilling cannot generate the curvature that we measured.