

SCALING MODULUS AS A DEGREE OF FREEDOM IN THE DESIGN OF LOCUST LEGS

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Summary

Previous work has shown that the scaling of mechanical behaviour in bending of the metathoracic tibiae of the African desert locust (*Schistocerca gregaria*) is not predicted by the scaling of external dimensions. The flexural stiffness of the tibia scales to (body mass)^{1.53}, which is similar to the predictions of the elastic similarity model of scaling. The external dimensions, however, scale in a manner that produces relatively more elongate limb segments – an observation that differs from the predictions of any existing scaling model. In this paper, we examined two alternative hypotheses to explain this uncoupling of morphology and mechanics: (1) that the load-bearing cuticular material is distributed in the legs in a manner that is not indicated by changes in external dimensions, or (2) that the stiffness of the cuticular material is altered to produce the observed scaling of flexural stiffness.

The second moment of area (I) scaled to (body mass)^{1.19}, which was similar to scaling I to (tibial radius)⁴. This indicates that the relationship between the external dimensions of the tibiae and the specific distribution of load-bearing material is conserved independently of scale. Therefore, the locust achieves the observed scaling of flexural stiffness by altering the modulus of the load-bearing cuticular material. In fact, the time-dependent modulus (E') scales to (body mass)^{0.311}. In essence, the scaled material stiffness provides a degree of freedom in design in addition to external morphological dimensions in accommodating the changing demands placed on a skeletal structure with increases in body size.

Introduction

Skeletal scaling demonstrates the relationship between shape and size in the structural design of a skeleton. Specific scaling models, or allometries, have been developed that predict how morphology might change to meet the changing mechanical demands of increasing body size (see, for example, McMahon, 1973, 1975). The various predictions of such models are based on the belief that one or more characteristic external dimensions of a skeletal structure can tell us about the mechanical behaviour of the structure and, therefore,

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that the scaling of external dimensions will reflect the scaling of mechanical behaviour. This belief is only valid if the structures being considered are constructed from materials that exhibit scale-independent mechanical properties and if the geometric distribution of the materials is also scale-independent. If true, then the characteristic external dimensions are the principal degrees of freedom in the design of a given skeletal structure.

Previous work performed in our laboratory has demonstrated that the scaling of external dimensions of the locust's legs does not predict the scaling of the mechanical behaviour of the legs in bending. The scaling of the locust metathoracic ('jumping') tibiae's external dimensions produces legs that increase in length faster than they increase in diameter (i.e. becoming increasingly slender) as the animal becomes larger. Engineering theory predicts that this changing geometry will result in legs that are subject to relatively larger deformations in larger animals. Mechanical testing revealed, however, that the legs are constructed in a manner that produces normalized deformations (i.e. deflections per unit length of beam per unit body mass) that are scale-independent. That is, the mechanical behaviour approximated the predictions of the elastic similarity model (McMahon, 1975), even though this model predicts that limb dimensions will scale in a manner that produces increasingly stout limbs in larger animals. Thus, the scaling of external dimensions does not provide sufficient degrees of freedom to explain fully the scaling of the locust's legs. In this paper, we will examine the nature of this 'uncoupling' between the scaling of dimensions and the scaling of mechanical properties in locust legs.

We have analyzed the mechanical behaviour of the locust tibiae in bending, but what is the relationship between the morphology of a bending tibia and its mechanical behaviour? We can calculate the deformation of a cantilever beam of specific dimensions loaded at its end with the following formula:

$$d = Fl^3/3EI, \quad (1)$$

where the deflection (d) of a cantilever is equal to the force applied (F) times the length of the beam (l) cubed, divided by three times the flexural stiffness (EI) (Wainwright *et al.* 1976). The flexural stiffness of a unit length of beam is its resistance to deformation under a given bending load and is composed of two parts. E is the Young's modulus of elasticity of the material from which the beam is made, and I is the second moment of area of the beam – a variable that accounts for how much material is present and the geometry of its distribution (Gordon, 1978). In our previous work, we estimated the flexural stiffness of the tibia using a dynamic testing technique that produced a flexural storage stiffness ($E'I$), composed of a time-dependent material stiffness E' , and a time-independent I (adapted from Ferry, 1980; see Katz and Gosline, 1992). We observed that $E'I$ scaled to (body mass)^{1.53} in the set of legs that were tested (Katz and Gosline, 1992).

We have speculated that the uncoupling between morphological and mechanical characters that we have observed in *Schistocerca gregaria* could be the result of one or both of two processes: either the second moment of area of the legs could be related to the external dimensions in ways that are different from the geometrical analysis of more familiar objects, or the material stiffness of the cuticle could be changing to provide a degree of freedom, in addition to linear morphological dimensions, in the design of the skeleton in accommodating increasing body size.

With respect to the deployment of the first of these strategies, it is possible that the distribution of cuticle may change across instars, resulting in a change in I that is not reflected in a change in the externally measured diameter. The exact formula for I is:

$$I = \int_{Y_{\max \text{ in compression}}}^{Y_{\max \text{ in tension}}} y^2 dA, \quad (2)$$

where dA is the increment of cross-sectional area located a distance y away from the neutral axis, a line through the centroid of the cross section normal to the bending moment (Gordon, 1978). This integral of the product of an area and a distance squared results in units of distance to the fourth power. We can, therefore, relate the stiffness of a beam to the fourth power of its radius. In the case of locust legs, the radius scales to body mass raised to the 0.311 power (Katz and Gosline, 1992). This predicts that I will scale to body mass raised to the 1.244 power. Since $E'I$ scales to body mass raised to the 1.53 power (Katz and Gosline, 1992), we might hypothesize that the observed scaling of flexural stiffness represents a strategy that does not conserve the relationship between geometry and dimension and scales I to body mass raised to a power other than 1.244. Because the cuticular leg segments of locusts are thin-walled cylinders, a small adjustment in the distribution of material on the *inside* of the cross section of a locust leg could impart large changes in mechanical properties that are not easily inferred from measurement of external dimensions.

The second strategy is that the E' of the cuticle material is altered to accommodate changes in morphology of the limb segment in order to maintain an elastically similar flexural stiffness. It has been observed that, within an instar, the modulus increases during the immediate post-moult period of sclerotization as a result of dehydration (Hepburn and Joffe, 1974a; Vincent and Hillerton, 1979; Vincent, 1980). It may be that the material properties of the cuticle are different in different instars. Hepburn and Joffe (1974b) observed for *Locusta migratoria* that the structural (i.e. spring) stiffness of its exoskeleton relative to body mass remains constant across instars, and they suggest that this indicates that the material stiffness of the tanned cuticular material is constant through the life history.

In this study, we have examined this uncoupling of mechanical behaviour and morphology explicitly by measuring the second moments of area of the same legs that were tested in bending, and teasing apart the relative contributions of material stiffness (E') and distribution of material (I) to the scaling of flexural stiffness ($E'I$). The data indicate that the locust increases the value of E' by about fivefold over its lifetime, while maintaining a consistent relationship between the fourth power of tibial radius and I , in order to achieve the observed scaling of flexural stiffness.

Materials and methods

Because of the labour-intensive nature of specimen preparation, we chose to examine a subset of legs to represent the population that was tested previously (Katz and Gosline, 1992). Therefore, methods for animal husbandry, morphological measurement and mechanical testing are exactly the same. Specimens for measurement were chosen to represent the range of body masses covered in the life of the locust. Twenty-one first and

second instars were chosen to represent small locusts, seven fourth instars were used to provide intermediate body sizes, and sixteen adults were used to provide values for the large animals. Additionally, fifty-six fifth-instar samples were used to show how the changes in second moment of area occur within an instar. Because the cuticle is soft immediately after a moult, we excluded all first day of instar individuals from the scaling analysis (see Discussion).

Following three-point mechanical testing described previously (Katz and Gosline, 1992), test pieces were placed in 3.7% formaldehyde solution for fixation and stored for later embedding and sectioning. Each tibia was dehydrated in an alcohol series and embedded. The technique used to produce cross sections of the locust legs was different in the large and the small specimens. Legs of first and second instars were embedded in paraffin and sectioned with a microtome at a thickness of 20 μm . Older locusts' legs were embedded in Araldite epoxy. Each Araldite block was sectioned at 800 μm with a bone saw. These 800 μm thick sections were glued to a glass slide with Araldite and ground to approximately 125 μm with alumina sandpaper of decreasing grit size. The thickness of the section was controlled by placing parallel strips of 0.005" steel shim stock between the microscope slide and the sandpaper. The surfaces were then polished with 600 grit polishing paper. Optical imperfections in the surface of the specimens were filtered out by placing a drop of immersion oil on the section and then covering it with a glass coverslip. Sections used for measurement were chosen from the approximate mid-shaft location on the tibia. The leg sections formed two sets: the first had no apparent distortions of profile associated with spurs and the I calculated for these sections was used to estimate E' ; the second set demonstrated geometry associated with the spurs and these were used to determine the potential influence of spurs on stiffness (see Discussion below). The cross sections of the tibiae were observed with a Leitz Orthoplan-Pol microscope fitted with a video camera.

Images of the legs' cross sections were captured from the video signal with a video frame-grabbing computer interface (PIP 1024B, Matrox Electronic Systems Ltd, Dorval, Quebec, Canada) and stored in computer memory as a 256-greyscale bit-mapped image. Editing and analysis of the video images was accomplished with the V video image-processing system (Digital Optics Ltd, Auckland, New Zealand) on a PC-type computer. Each 256-greyscale video image (Fig. 1A) was manually analyzed to detect the inner and outer perimeter of the entire cuticle and the endocuticular-exocuticular boundary. The image-processing software was then used to set a threshold greyscale level that defined the leg cross sections against the background, producing binary images. The perimeter of the endocuticle was produced by arithmetically subtracting the image of the exocuticle from that of the entire cuticle (Fig. 1B,C,D). The image-analysis software then integrated each of the video images to calculate the second moment of area of the cross sections that represented the entire cuticle, the exocuticular component and the endocuticular component for each leg that was successfully sectioned.

The diameters of 20 selected tibial sections, measured with the video analysis system, showed less than a $\pm 3\%$ difference from values obtained previously with a filar micrometer eyepiece for the same specimens (Katz and Gosline, 1992). Dimensional changes that resulted from the histological process were, therefore, ignored.

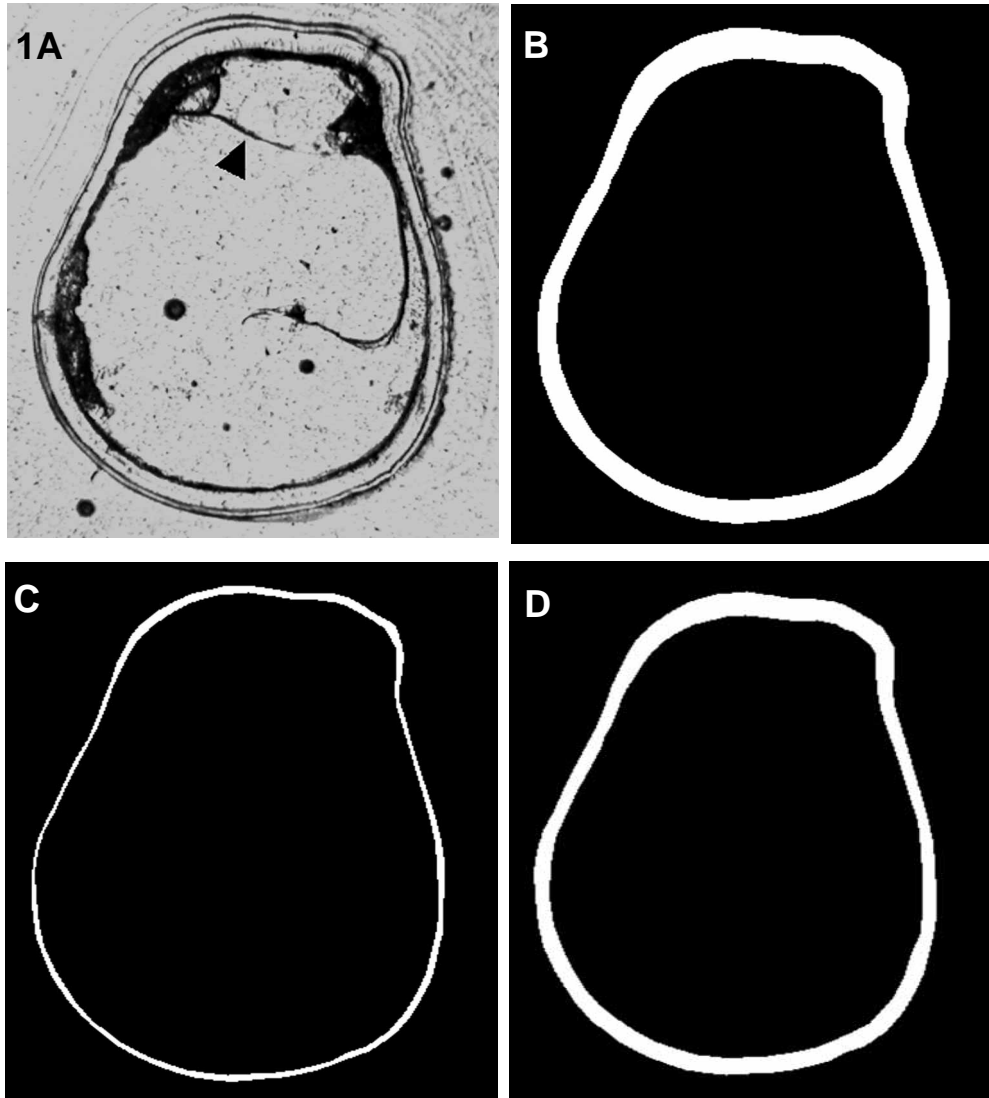


Fig. 1. (A) Typical cross section from a fifth-instar locust metatibia. The top of the picture is the anterior direction. For scale, the diameter of the section in the anterior–posterior direction is 2.52 mm. The arrowhead indicates the edge of the membranous connection mentioned in the text as a possible tensile stay to control the cross-sectional shape of the tibia during the initial post-moult period. (B) Example of the section shown in A processed for calculation of I for the entire cuticle. The inner and outer margins of the image in A have been defined manually, and a binary image has been created with the section white and the background black. The image-analysis software performs the calculation on the binary image by summing over all white pixels. (C) The same section with the margins defined only for the exocuticle. (D) The same section with the margins defined only for the endocuticle.

After locating the position of the centroid of the cross-section (x_c, y_c) , the image-analysis software computes the second moment of area with the following summation:

$$I_{yy} = \sum_y \sum_x (y - y_c)^2 dA . \quad (3)$$

The image-analysis software performs this summation numerically as the sum of pixel elements (dA) in each line of the bit-map, weighted by the square of their distance from the centroid $[(y - y_c)^2]$. The values are then summed over all the lines of the bit-map image from the location of the centroid out to the margin of the section (\sum_y). The program also calculated the second moment of area in the orthogonal direction (I_{xx}) as:

$$I_{xx} = \sum_x \sum_y (x - x_c)^2 dA , \quad (4)$$

and a related moment, the product moment (I_{xy}) as:

$$I_{xy} = \sum_y \sum_x (x \times y) dA . \quad (5)$$

Together, these moments determine the aspect ratio, or ellipticity, of the leg cross section and thus describe the distribution of the material in the section (Young, 1989).

Each measured value of $E'I$ was divided by the measured value of I for that leg to estimate the value of E' for each specimen. Because the measured I represents one point along a beam of non-uniform shape, and because the cuticle itself is composed of a composite laminate of potentially inhomogeneous character, the value we report for E' represents a best estimate of the material properties of the cuticle.

As mentioned previously, material at the margins of the cross section contributes greatly to the stiffness of the test piece. As such, orientation of the non-circular cross sections, both in the mechanical testing and in the measurement of I , can have significant effects on the results. To control for the role of orientation, a subsample of images was analyzed for the changes in I that result from rotation of the neutral axis. By calculating the three moments of inertia for a subsample of legs, we were able to estimate I for any orientation of the neutral axis (θ) using the following relationship (Young, 1989):

$$I_\theta = I_{yy} \cos^2 \theta + I_{xx} \sin^2 \theta - I_{xy} \sin 2\theta . \quad (6)$$

Thus, by varying θ through a range of angles, we were able to characterize the sensitivity of the principal second moment of area (I_{yy}), and thus the maximum bending stiffness of the leg section, to changes in the orientation of the neutral axis. To minimize confusion over the various moments of area, I without subscript will refer to the principal, or largest, moment that we calculated for a given leg section or sections.

Results

Changes in I within the fifth instar

The time course of changes in flexural stiffness for the sub-population of fifth-instar locust legs used in this study are shown in Fig. 2. $E'I$ averaged a low value of

approximately 10^{-6} N m^2 on the first day of the instar, and increased by about 30-fold by the second day. Thereafter, $E'I$ remained relatively constant at a value of approximately $4 \times 10^{-5} \text{ N m}^2$. We feel that the large variation on the first day reflects our uncertainty in ageing of the animals (\pm half a day).

The relative contributions of the cuticular components are shown in Fig. 3. Values of I for the exocuticle alone had a relatively high mean value of $3.6 \times 10^{-15} \text{ m}^4$ on the first day of the fifth instar and remained close to this value for the remainder of the time spent in this instar. The endocuticular component started out at a low mean value of $1.9 \times 10^{-16} \text{ m}^4$, but increased asymptotically until approximately day five, where it attained a mean value of $8.6 \times 10^{-15} \text{ m}^4$. The values of I for endocuticle on the first day of the fifth instar had a large variance, as one of the samples had almost no measurable endocuticle. Examination of the actual cross sections of the tibiae suggested that, immediately after the moult, there was no measurable endocuticle, and those samples that were taken on the first day that contributed to the non-zero estimate of endocuticular I reflected the uncertainty in the measurement of age.

Significantly, there appeared to be no detectable breakdown of the endocuticular material that could be correlated with apolysis on or about day five. However, it was observed that after day five there was a clear separation of the endocuticle from the underlying epithelial layer. Indeed, in some samples it was also possible to observe the next stage's tibia folded up within the lumen of the tibiae, indicating that the events that are associated with apolysis and the production of the next stage's exoskeleton had occurred without reducing the second moment of area of the endocuticular material.

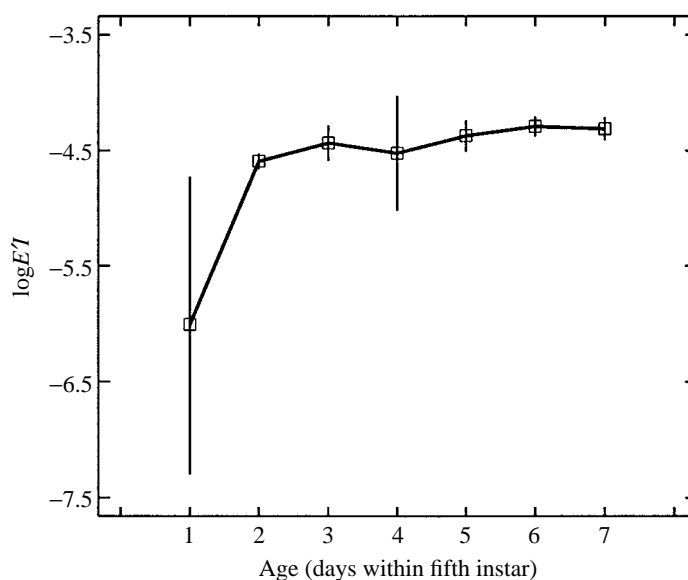


Fig. 2. The relationship between the logarithm of flexural stiffness ($E'I$ in N m^2) and age within the fifth instar. Data points are mean values and bars show standard errors of the mean. Age is reported as days within the instar.

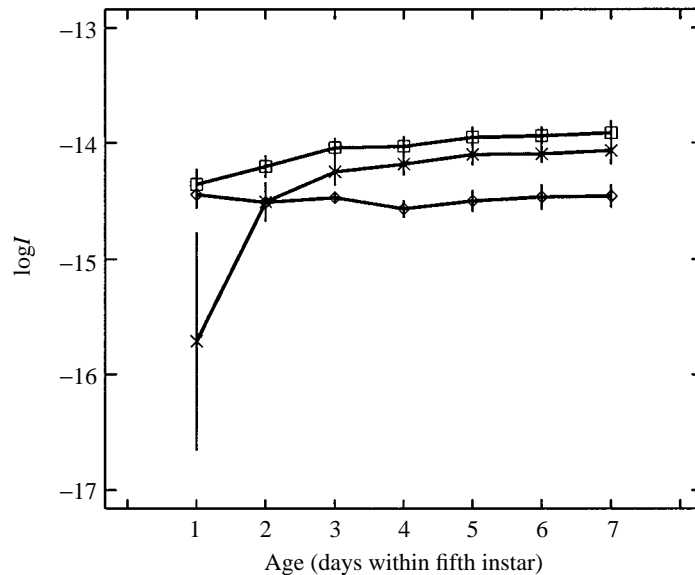


Fig. 3. The relationship between the logarithm of second moment of area (I in m^4) for the exocuticular (\diamond), endocuticular (\times) and entire cuticle (\square) components and age within the fifth instar. Data points are mean values and bars show standard errors of the mean.

The time course of changes in I for the entire cuticle is also plotted in Fig. 3. As this variable is the arithmetic sum of I calculated for each of the components, it demonstrates a small increase from a mean value of $4.38 \times 10^{-15} \text{m}^4$ on day one to a mean of $1.22 \times 10^{-14} \text{m}^4$ on day three and then remains fairly constant for the remainder of the instar.

Scaling across instars

In the sample of locust legs used in this analysis, the flexural stiffness of the legs scaled to body mass raised to the 1.505 power (s.e.=0.030, $r^2=0.966$). This slope is not significantly different from the slope of 1.532 ($t_s=0.342$, d.f.=513, $P<0.05$) for the entire sample reported by Katz and Gosline (1992). This indicates that the data used in this analysis represent the population as well as do those described previously.

Fig. 4 shows the scaling relationships for the second moment of area for the various components of the cuticle with increases in body mass. I of the exocuticular component of the tibiae scaled to body mass raised to the 1.090 power (s.e.=0.026, $r^2=0.952$). I of the endocuticular component scaled to body mass raised to the 1.258 power (s.e.=0.031, $r^2=0.949$). I calculated for the entire cuticle scaled to body mass raised to the 1.195 power (s.e.=0.027, $r^2=0.957$).

By normalizing the measured values of $E'I$ by the calculated value of I for whole cuticle, we can estimate an average modulus of elasticity, E' , for the tibial material. In the absence of specific data on the relative stiffnesses of the exo- and endocuticular materials, we are not able to calculate separate moduli for each cuticular component. Values of E'

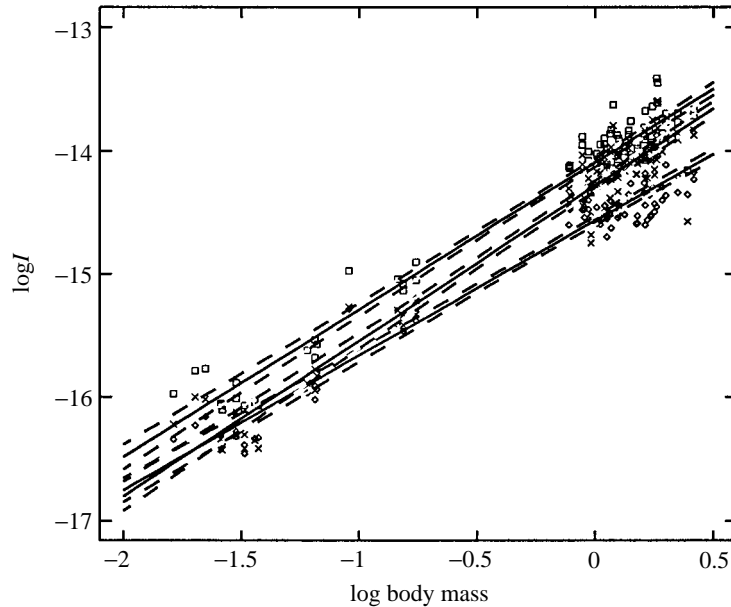


Fig. 4. The relationship between the logarithm of second moment of area (I in m^4) for the exocuticular (\diamond), endocuticular (\times) and entire cuticle components (\square) and the logarithm of body mass (in g). The equation of the regression of exocuticular I on body mass (bottom line) was $y = -14.569 + 1.090x$ ($F_s = 1738.74$, d.f.=1, 89, $r^2 = 0.9523$). The equation of the regression of endocuticular I on body mass (middle line) was $y = -14.283 + 1.258x$ ($F_s = 1611.19$, d.f.=1, 89, $r^2 = 0.9488$). The equation of the regression for the entire cuticle's I on body mass (top line) was $y = -14.092 + 1.195x$ ($F_s = 2016.63$, d.f.=1, 89, $r^2 = 0.9568$). Dashed lines show 95 % confidence intervals of the regression lines.

ranged from a mean value in first instars of $8.5 \times 10^8 \text{ N m}^{-2}$ (s.e. = $1.4 \times 10^8 \text{ N m}^{-2}$) to a mean value in adults of $4.6 \times 10^9 \text{ N m}^{-2}$ (s.e. = $7.0 \times 10^8 \text{ N m}^{-2}$). Although this mean value of E' for adults is somewhat lower than the value of $9.4 \times 10^9 \text{ N m}^{-2}$ reported by Jensen and Weis-Fogh (1962), inspection of the data in Fig. 5 shows that their value is within the range of values that we observed for adults.

Fig. 5 also demonstrates the scaling relationship of this average E' with increases in body mass. E' scales to body mass raised to the 0.311 power (s.e. = 0.033, $r^2 = 0.511$). This slope was significantly greater than a slope of zero ($t_s = 9.541$, d.f. = 89, $P > 0.05$).

Orientation of the neutral axis

The shapes of the tibial cross sections are not circular or even strictly elliptical. They form a very characteristic shape that imparts potentially interesting mechanical behaviour. Fig. 1A shows a representative cross section from a fifth-instar locust's tibia. The relatively circular posterior margin and relatively thicker-walled corners on the anterior lateral margins are characteristic. The necking that produces the concavity at approximately the one-quarter to one-third point from the anterior to the posterior margin co-occurs with a membranous connection that spans the lumen of the tibia. Immediately

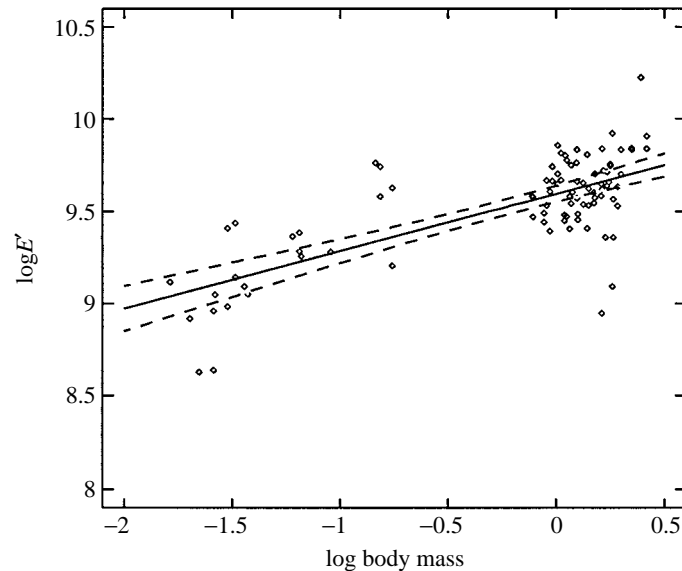


Fig. 5. The relationship between the logarithm of tensile modulus (E' in Nm^{-2}) and the logarithm of body mass (in g). The equation of the regression of E' on body mass was $y=9.596+0.311x$ ($F_8=91.040$, d.f. =1, 89, $r^2=0.5113$). E' was calculated for the entire cuticle treated as a homogeneous and continuous structure; see text for discussion. Dashed lines show the 95 % confidence intervals of the regression lines.

after a moult, when the cuticle is relatively soft, this membrane may provide a tensile stay that prevents the tibia's cross sections from becoming circular as the cuticle hardens over the initial 24 h.

Fig. 6 shows how the value of I for the cross section of six legs changes as the neutral axis is rotated through a range of angles from $+90^\circ$ to -90° . The mean values for I drop by an average value of only 4.54 % when rotated through $\pm 20^\circ$. We feel that this represents a relative insensitivity to orientation, and indicates that our measures of both $E'I$ and I are reasonably uninfluenced by orientation of the neutral axis.

Thus far, we have talked about cross sections that were free of any morphological feature that was associated with spurs, but spurs do exist and their contribution to the mechanical behaviour of the tibiae must be considered. The presence of spurs produces a bilaterally asymmetrical distortion in the relatively circular perimeter of cuticle along the posterior margin of the tibial cross sections. The distortion takes the form of a bump that puts more material away from the neutral axis on alternating sides of the tibiae. Presumably, this distribution of material increases I and shifts the orientation of maximum stiffness towards alternate sides of the tibiae. This could result in an average behaviour that has a local minimum of stiffness along the anterior-posterior axis.

Fig. 7A shows a figure of a tibial section with a spur on one side. Fig. 7B shows the binary image from this section, and Fig. 7C shows this image reflected through the anterior-posterior axis. Fig. 8 (open symbols) shows the relative I for the images in Fig. 7B and C as the neutral axis is rotated. It is noteworthy that the orientation of the

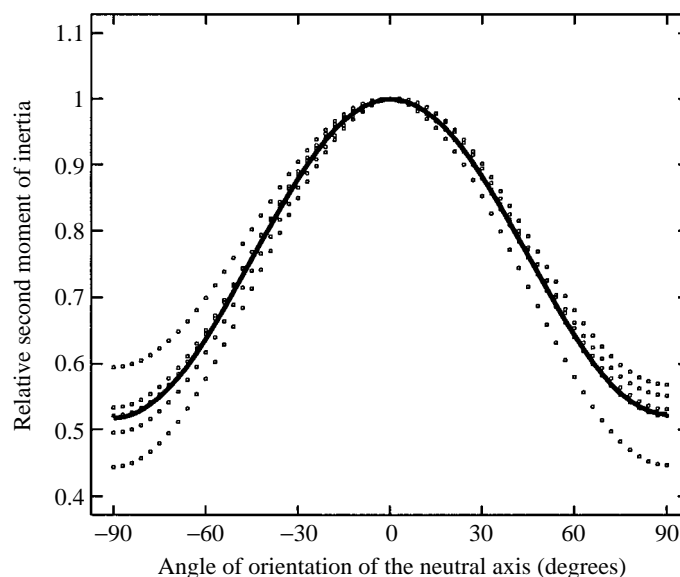


Fig. 6. Plot of the relationship between I and the orientation of the neutral axis for a sample of six legs (\square) and their average value at each orientation (solid line). Values of I are given relative to that at an angle of 0° .

principal moment is shifted away from the anterior–posterior axis by approximately 25° in sections with spurs. Thus, as one travels down the length of the leg, the direction of principal stiffness oscillates to the left and to the right of the midline. The possible role that spurs play in stabilizing the tibia in bending is discussed below.

Discussion

Changes in I within the fifth instar

The data for changes in I within the fifth instar seem somewhat equivocal on the question of the relative contributions of the two cuticular components towards whole-tibial stiffness. Over the first 3 days, the rapid increase in endocuticle matches the rapid increase in flexural stiffness. If the material stiffness is constant over that period, these results would suggest that the stiffness demonstrated by the whole tibia resides largely in the endocuticular component. Over the final 5 days of the instar, the flexural stiffness seems no more reflective of changes in I in the exocuticle than in the endocuticle, obscuring which cuticular component is making the most significant contribution to flexural stiffness. These apparently ambiguous inferences can be accepted if we recognise that, in the initial post-moult period, two processes occur: (1) an endocuticular layer is deposited, and (2) the preformed exocuticular layer becomes stiffer.

The data of Hepburn and Joffe (1974a) indicate that during the first 24–36 h the cuticle, which over that period is almost entirely exocuticle, increases in modulus by almost an order of magnitude. If so, then we cannot really depend on the correlation between I and $E'I$ to provide information on the relative contributions of the cuticular components to

tibial stiffness. From information provided by Zacharuk (1976) and Queathum (1991) on the effect of apolysis on the endocuticle, it was anticipated that changes in the endocuticle would be uncorrelated with changes in $E'I$. The fact that this was not the case prevents us from being able to discriminate the contributions of the cuticular components on the basis of our data on fifth-instar locusts. For this reason, in estimating the tensile modulus we

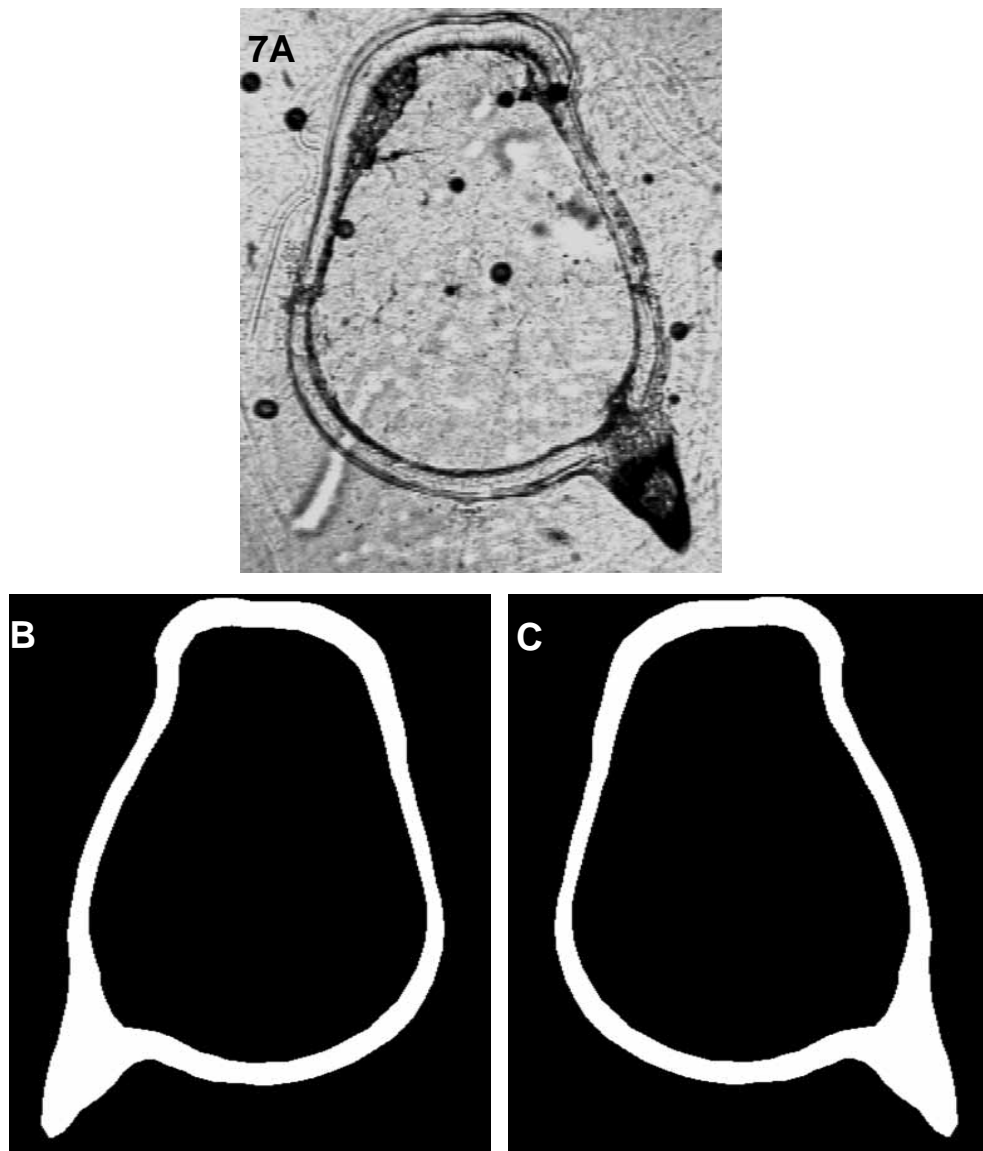


Fig. 7. (A) Cross section from the same leg as shown in Fig. 1A with the base of a spur projecting from the posterior margin of the section. (B) Example of the section shown in A processed for calculation of I for the entire cuticle in the same manner as in Fig. 1B. (C) The same image as in B, but reflected through its anterior-posterior axis.

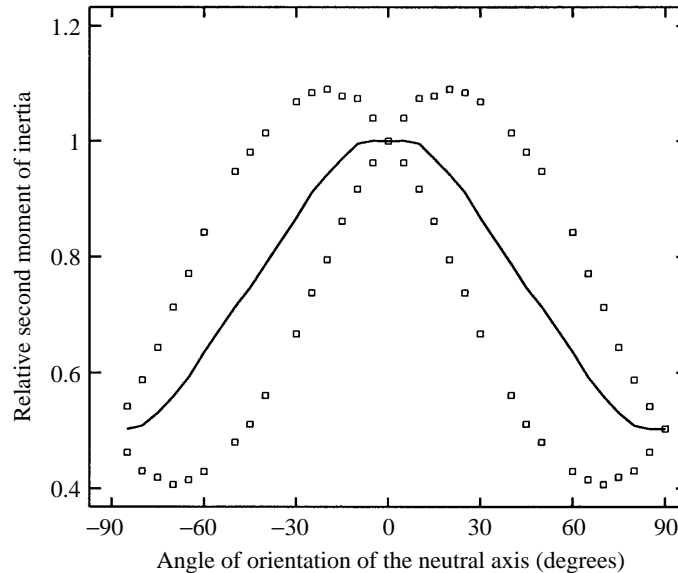


Fig. 8. Plot of the relationship between I and the orientation of the neutral axis for a tibial cross section with a spur (Fig. 7B) and its mirror image (Fig. 7C) (□), and for the 'average behaviour' (solid line) expected for the tibia over its entire length. Values of I are given relative to the 'average' value at an angle of 0° .

will consider the exocuticle and endocuticle to be homogeneous and continuous components, as did Jensen and Weis-Fogh (1962). As a result, the modulus values that are estimated represent lumped parameters for entire cuticle, and the stiffness for each component may be somewhat different.

Scaling

In previous work, we observed that if I is strictly a function of the fourth power of the diameter of the limb segment, which itself scales to body mass raised to the 0.311 power, then I should scale to body mass raised to the 1.244 power. Our observation that I for the entire cuticle scales to body mass raised to the 1.195 power is not significantly different from that prediction ($t_s=1.862$, d.f.=91, $P<0.05$). This provides an answer to our original question of the nature of the observed uncoupling of morphology and mechanical properties. The locusts are achieving elastically similar flexural stiffness by scaling the material stiffness of the cuticle, rather than applying an allometric scaling to I by changing the relative distribution of material in the cross section. This conclusion is demonstrated explicitly in Fig. 5, where E' is shown to scale to mass raised to the 0.311 power. It is intriguing to see such a distinct commitment to one of these alternative strategies, rather than a partial employment of both. One is tempted to hypothesize that there are constraints that determine the commitment to the observed programme which maintains the size-independence of the relationship between shape and dimension. Such a constraint might be associated with the developmental programme controlling the manufacture of these tubular members.

When compared with morphological parameters, the relationship between E' and body mass has relatively poor statistical strength. This is perhaps not surprising, as the variance in these data is the product of the variability in the mechanical measurement of $E'I$ and the variability in estimating I . Still, the relationship is highly significant ($F_s=91.04$, d.f.=1, 89) as a descriptor of the data. Therefore, we are confident that the elastic stiffness of the cuticle material is being treated as a scaled commodity in the design of the locust's legs.

For the endocuticular and exocuticular components of the cuticle, I is scaled to body mass differently. However, we have treated the cuticle as homogeneous in material properties, resulting in a preliminary conclusion that the modulus is a scaled commodity. An alternative hypothesis is that the modulus for each component is different and scale-independent, and that the amount of each component is varied to produce the average behaviour that is observed for entire tibiae. If this were true, we could estimate how different in modulus the components would have to be to produce the lumped behaviour. We believe this alternative hypothesis can be rejected by considering the limits to this design strategy. The hypothetical limiting cases are where one or the other component has a stiffness of zero, and the scaling of the average system is strictly a reflection of the scaling of I of the component that has non-zero stiffness. That is, we want to know whether we can get the entire structure's $E'I$ to scale to (body mass)^{1.53} given the following constraints: (1) the I of the endocuticular component scales I to (body mass)^{1.258}, (2) the exocuticular component scales I to (body mass)^{1.090}, and (3) the modulus of each material is scale-independent but potentially different for the two components. The answer would seem to be no. Even if the modulus of the exocuticle were zero, the entire cuticle's $E'I$ would only scale to (body mass)^{1.258}. Therefore, even if we accept the unreasonable assumption of zero stiffness for the exocuticle, we are forced to conclude that the modulus of the cuticular components is altered in response to changes in body size.

Ontogenetic scaling of material properties in skeletons

It is common for mammals to show substantial increases in material stiffness of bone during ontogeny (e.g. polar bear, Brear *et al.* 1990, and jack rabbits, Carrier, 1983). Is it fair, then, to make the claim that the changes in locust cuticle stiffness are a scaling design response separate from a normal ontogenetic process? As we have previously argued (Katz and Gosline, 1992, 1993), we believe the answer is yes. Gould (1975) has pointed out that allometric regression is a 'criterion of subtraction', meaning that the regression line provides information on the influence of body mass independent of any specific adaptation of animals at any specific point in the range of body masses. Thus, an observed allometry may hide, or may be influenced by, fundamental differences between large animals and small ones that are not strictly structural design consequences of body size. This we think is not the case for locusts. The accelerations produced in the jumps of first-instar locusts are as high as at any point in the animal's life history. Additionally, the velocities generated and the ballistic distances travelled are similar during the entire flightless portion of the life history (Katz and Gosline, 1993). The scaling of modulus for locust legs seems, therefore, to be different from that for the vertebrates that have been

examined in this way (i.e. jackrabbits, Carrier, 1983) in that these other animals are not competent locomotors during the early parts of their life history when the material stiffness of their bones is lower. Thus, by Gould's criterion, an ontogenetic scaling comparison within a vertebrate species would be unfair, as it would obscure functional differences in small and large skeletal designs. In locust ontogeny, however, the modulus is changing in functionally equivalent hoppers, and thus a criterion of subtraction may be fairly applied. In the locust, the modulus is 'scaling' to provide an additional degree of freedom in adjusting morphology to accommodate increasing loads with increasing size.

Given a design strategy that tries to keep a constant deformation per unit length with increasing size, the non-mineralized skeleton of the locust can adopt a morphology that is not predicted by the elastic similarity model. Now we know how the uncoupling between morphology and mechanical behaviour is accomplished, but it remains to be seen why it is uncoupled. Indeed, asking why may presuppose that the morphology demonstrated in the locust leg is an adaptation to a specific design issue. In subsequent papers, we hope to examine the hypothesis that this morphological developmental programme has a functional role in improving jump performance.

We do not yet know the specific nature of the changes that we have observed in E' . The cuticle is a fibre-reinforced composite material (Neville, 1975), and there are a number of ways that these materials can be modified (Wainwright *et al.* 1976). It would be interesting to investigate whether the animals are altering the protein polymer that glues the crystalline chitin fibres together, by altering hydration or cross-link density perhaps, or whether the volume fraction and/or the orientation of the crystalline fibres are altered.

Orientation of the neutral axis

One can imagine that some fraction of the jumps that a locust makes in its lifetime are made from morphologically complex surfaces. As a result, it is also possible that the loads encountered during the jump impulse will not be applied co-linearly with the anterior-posterior axis. Given that the jump impulse produces large deflections in the tibia (Katz and Gosline, 1992; Brown, 1963), and that we have observed the tibia to buckle when animals are restrained while jumping, such off-axis loading may have important functional consequences. The observation in Fig. 8 that spurs generate an oscillation in the direction of the principal moment in the tibia suggests an interesting possibility, that the cross-sectional shape of the tibia provides a stability to off-axis loads experienced by the limbs.

Any beam whose cross-sectional shape is uniform along its length cannot have more than one orientation of maximal I (equation 6). If the tibia had the same cross section along its entire length, then the relationship between I and the orientation of the neutral axis would be similar to that shown in Fig. 6 at all points. Any off-axis loading would tend to flex the leg in a direction towards the axis of lower stiffness.

The solid line in Fig. 8 illustrates the possibility that the oscillation of the direction of principal stiffness along the tibia might on average create a beam with a broad range (± 10 – 15°) of relatively constant stiffness, or even a local minimum, that might impart a considerable improvement in stability. The solid line was derived by calculating the arithmetic average of the left- and right-spur sections (open symbols), and is equivalent to

saying that the behaviour of the beam averaged along its entire length will be the average of the behaviour of the two extremes.

The influence of spurs on the flexural stiffness of the tibia may be more significant than the composite behaviour estimated above. Within each segment of the leg that has a spur, the primary loading will be off the axis of greatest stiffness. As a result, each local segment of leg will be subject to flexion in a direction away from its orientation of maximum stiffness and towards the anterior–posterior axis. The magnitude of the flexion in this small length of leg will itself be small because of the large dependence of deflection in a beam on length (Gordon, 1978). Furthermore, distal to each small segment of leg, there will be a similar small segment loaded in the opposite direction because it has a spur on the other side. It may turn out that this alternating pattern of laterally flexed short beams produces greater stability than the solid line in Fig. 8 suggests.

We would prefer not to draw too strong a conclusion from the analysis of the shape of this length-averaged or composite cross section for three reasons. First, it is not clear that the mechanical properties of the tibial wall are homogeneous between the inter-spur and spur cuticle; second, it might be purely coincidental that a spur which may be developed as an anti-predator mechanism also influences mechanical behaviour in an externally evaluated positive manner; and third, it is possible that spurs act to produce stress concentrations (e.g. from torsional moments) that would compromise any improvement in stability derived from their presence. Bertram and Biewener's (1988) model for pre-bend in vertebrate long bones was constructed around such a compromise. The bones are predicted to be pre-bent only when the value of loading predictability outweighs the cost inherent in increased bending moments. Without more detailed knowledge of the consequences of spur formation on the local loading of the cuticle, we are not prepared to commit ourselves to an interpretation of the mechanical role of the spurs in stabilizing the bending deformation of the tibia other than to advance the hypothesis that the shape of the leg cross section, and perhaps the presence of the spurs, will act to provide a degree of stability in the loading of the tibia during the jump impulse.

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