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RNAi of the elastomeric protein resilin reduces jump velocity and resilience to damage in locusts

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Resilin, an elastomeric protein with remarkable physical properties that outperforms synthetic rubbers, is a near-ubiquitous feature of the power amplification mechanisms used by jumping insects. Catapult-like mechanisms, which incorporate elastic energy stores formed from a composite of stiff cuticle and resilin, are frequently used by insects to translate slow muscle contractions into rapid-release recoil movements. The precise role of resilin in these jumping mechanisms remains unclear, however. We used RNAi to reduce resilin deposition in the principal energy-storing springs of the desert locust (*Schistocerca gregaria*) before measuring jumping performance. Knockdown reduced the amount of resilin-associated fluorescence in the semilunar processes (SLPs) by 44% and reduced the cross-sectional area of the tendons of the hind leg extensor-tibiae muscle by 31%. This affected jumping in three ways: First, take-off velocity was reduced by 15% in knockdown animals, which could be explained by a change in the extrinsic stiffness of the extensor-tibiae tendon caused by the decrease in its cross-sectional area. Second, knockdown resulted in permanent breakages in the hind legs of 29% of knockdown locusts as tested by electrical stimulation of the extensor muscle, but none in controls. Third, knockdown locusts exhibited a greater decline in distance jumped when made to jump in rapid succession than did controls. We conclude that stiff cuticle acts as the principal elastic energy store for insect jumping, while resilin protects these more brittle structures against breakage from repeated use.

elastic energy storage | biomechanics | insect locomotion | muscle-spring interaction

Significance

The extraordinary jumping abilities of insects like grasshoppers and fleas rely on the sudden release of stored elastic energy from catapult-like structures. Resilin, a rubber-like protein with outstanding resilience and durability, forms an integral part of these catapults but is its principal role to store elastic energy or to protect a stiffer cuticular spring? We experimentally reduced the amount of resilin using RNA interference (RNAi) in an iconic jumping insect, the desert locust. This knockdown caused only a modest decrease in jump take-off velocity but greatly increased the incidence of catastrophic breakages, suggesting that resilin's primary role is to protect stiffer, energy-storing cuticle with which it forms a composite matrix in catapult structures.

Elastomeric proteins help maintain the functional integrity of many biological structures that undergo repeated cycles of elastic deformation (1), such as those involved in jumping, a behavior used by many animals to increase the speed of locomotion, escape from predators, or initiate flight (2). Small animals, such as insects, need the propulsive movements powering jumps to be both rapid and forceful, a combination that is difficult to achieve using muscles acting on short levers, since the greatest forces are produced by low muscle strain rates (3–5). Small animals therefore commonly jump using catapult-like mechanisms, in which slow muscle contractions deform initially "latched" spring-like structures, which store energy without external limb movement (2, 3, 6, 7). In insects, these springs are made from highly modified cuticle of the body wall, leg joints, and/or tendons (apodemes). Release of the stored energy leads to a sudden recoil, causing rapid leg extension with high accelerations. The elastomeric protein resilin, in combination with stiff cuticle, is a ubiquitous feature of elastic energy stores in jumping insects such as fleas (Siphonaptera), true bugs (Hemiptera), and grasshoppers (Orthoptera) (3, 6, 8–19). Catapult mechanisms must deliver their stored energy repeatedly and reliably without becoming damaged (10, 20), but the precise role of resilin in fulfilling these requirements remains unclear.

Resilin has outstanding mechanical properties with a resilience (the extent of elastic recovery after deformation) of 92 to 97% and a fatigue limit of over 300 million cycles, outperforming synthetic rubbers (21–23). In dragonflies, the wing tendon is largely composed of resilin and can be stretched to 300% of its resting length, yet it shows almost perfect elastic recovery (24, 25), losing less than 5% of elastic energy even during oscillations at 200 Hz (26). These properties led to suggestions that resilin can act as a useful spring (12, 13). In fleas, for example, a pad of resilin in the thorax has been proposed as the principal energy store (12, 27, 28). More recent biomechanical analyses, however, have suggested that the low elastic modulus of resilin would require it to strain further than anatomically possible to store enough energy to power a jump (10, 11, 20), and calculations suggest that resilin may be capable of storing just 2% of the required energy in a biologically plausible setting (20). Instead, these studies suggest that the springs are complex composite structures of interwoven resilin and stiffer cuticle, with the latter acting as the principal energy store and

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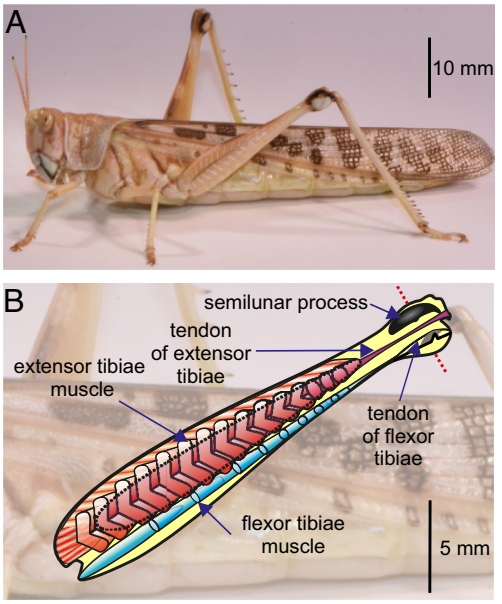

Fig. 1. Locust jumping anatomy. (*A*) An adult desert locust, *Schistocerca gregaria*. (*B*) Structures in the hind femur associated with jumping. The extensor- (red) and flexor- (blue) tibiae muscles cocontract, deforming the bow-like semilunar processes (SLPs) and stretching the extensor tendon (purple, shown as a dotted line within the extensor muscle), whose recoil powers the jump. The red dotted line denotes the level of the transverse section shown in Fig. 2.

resilin having a protective function. Experimental demonstrations of the role of resilin in the jumping of insects are, however, lacking.

We have analyzed the role of resilin in jumping using desert locusts (*Schistocerca gregaria*), which combine a well-characterized jumping mechanism with a particular sensitivity to RNA interference (RNAi) (29, 30). Locusts use their enlarged hind legs for jumping (8). Each hind femur contains a massive extensor-tibiae muscle and a much smaller flexor tibiae muscle, combined with extensive mechanical specializations of the femoro-tibial (FT) joint (Fig. 1*B*) (31). Prior to jumping, the tibia is fully flexed about the femur, which gives the flexor muscle a substantial mechanical advantage over the extensor muscle (17). Before jumping, both muscles cocontract for approximately 300 to 500 ms (18), during which muscle work is stored as elastic strain energy. The role of the flexor muscle is to hold the tibia in place while the contraction of the extensor muscle slowly distorts the principal energy stores (32), which contain extensive deposits of resilin (10, 33). The first energy store is the tendon (apodeme) of the extensor-tibiae muscle, which is placed under tension. As the tendon pulls, it compresses the distal FT joint, displacing the pivot in a proximal direction (8, 34), and in doing so, bends the second energy store, two bow-like semilunar processes (SLPs), specialized cuticular structures on either side of the femur close to its joint with the tibia. Approximately 50% of elastic energy is stored in the SLPs and 50% by the tendon, with a small amount stored in the femoral cuticle (8). Finally, the flexor tibiae muscle is relaxed, and the elastic recoil from the energy stores extends the tibia with high acceleration, powering the jump (18, 34).

To investigate the functional significance of resilin in the context of jumping, we used RNAi to strongly reduce the deposition of resilin in the energy storage structures, to then compare the jumping velocity and resistance to jump-inflicted damage of knockdown and control locusts.

Results

RNAi Reduced Resilin Deposition in the SLPs and Extensor-Tibiae Tendons. Our identified *S. gregaria pro-resilin* sequence obtained from the hind leg FT joint (GenBank accession code MZ147630) had all the established features of resilin (*SI Appendix*, Figs. S1 and S2), and qRT-PCR confirmed that its temporal expression pattern (*SI Appendix*, Fig. S3*A*) agreed with previous results (22, 33, 35). RNAi reduced *pro-resilin* mRNA by 98.1 ± 1.1 % compared with ds*GFP*-injected controls (mean ± SD; *SI Appendix*, Fig. S3*B*), with subsequent strong phenotypic effect on the amount of mature resilin present in the SLPs and extensor tendons of adults (Fig. 2). Body mass was not significantly affected by the RNAi (1.80 ± 0.34 g in controls vs 1.67 ± 0.38 g in ds*Res*; Fig. 2*B*; *t* test, t_{62} = 1.36, P = 0.18).

In transverse section, the SLP consists of a thick layer of cuticle with a projection into the lumen of the FT joint (Fig. 2 *A*, *C*, and *E*). UV-DAPI illumination revealed a vivid blue fluorescence, characteristic of resilin (33), at the medial face of the SLP (Fig. 2*C*), which decreased in intensity deeper within the structure (see transects in Fig. 2*E*). The external face of the SLP and a longitudinal ridge projecting internally from it consisted of dark cuticle that did not fluoresce. The total area of the SLP in mid transverse section did not differ between ds*Res* (0.054 ± 0.016 mm^2) and control locusts (0.057 ± 0.013 mm^2; t_{62} = 0.7, P = 0.48; Fig. 2*G*). The mean fluorescence within the SLP, however, as measured along two transects (Fig. 2*E*), was substantially less in ds*Res* locusts compared to controls (Fig. 2*H*; 20,207 ± 12,780 and 37,026 ± 10,399, respectively, a 44% decrease; t_{62} = 5.8, $P = 2.5 \times 10^{-7}$). The difference in the median fluorescence was greater still at 58% (38,066 in controls vs. 15,939 in ds*Res*; Fig. 2*H*). Examples of the weaker fluorescence in ds*Res* SLPs compared to controls are shown in Fig. 2*C*. We suggest that resilin in the SLP is embedded in a matrix of other material unaffected by the knockdown, resulting in an attenuated fluorescence when smaller amounts of resilin were distributed across similar volumes. This interpretation is supported by nanoindentation measurements of the indentation modulus across the SLP (*SI Appendix*, Fig. S4), which revealed no significant differences between ds*Res* and control locusts (ANOVA, $F_{1,96}$ = 1.9, P = 0.171), suggesting that its material properties are largely due to chitin and cuticular proteins other than embedded resilin.

Pro-resilin RNAi had a contrasting effect on the extensor tendon. (Fig. 2 *A*, *D*, *F*, *I*, and *J*). There was an average 31% decrease in tendon cross-sectional area at a mid-SLP level (Fig. 2*I*; 0.062 ± 0.017 mm^2 in controls compared to 0.043 ± 0.016 mm^2 in ds*Res* locusts; t_{60} = 4.5, $P = 3.7 \times 10^{-5}$), but little difference in the intensity of fluorescence as measured across a transverse transect (Fig. 2*F*; integrated density over 400 µm 47,556 ± 10,594 in control and 44,147 ± 12,634 in ds*Res* locusts; t_{60} = 1.1, P = 0.259; Fig. 2*J*), as shown by individual examples of control and ds*Res* tendons (Fig. 2*D*). Locust tendons are largely composed of resilin interspersed with thin chitinous lamellae (25, 36): reducing resilin expression therefore resulted in a decrease in tendon size.

***Pro-Resilin* RNAi Produced a Significant but Modest Reduction in Jump Velocity.** We recorded 5 jumps from each of 31 ds*Res* and 33 control locusts to then extract a maximum and mean take-off velocity per individual (see examples in Movie S1). The maximum take-off velocity was 15% slower in ds*Res* locusts compared to controls (2.16 ± 0.33 m s^{-1} compared to 2.54 ± 0.31 m s^{-1}; Fig. 3*A*; t_{62} = 4.7, $P = 1.4 \times 10^{-5}$). Likewise, the average take-off velocity was 18.9% lower in ds*Res* locusts than in controls (1.78 ± 0.33 m s^{-1} compared to 2.19 ± 0.32 m s^{-1}; *SI Appendix*, Fig. S5*A*; t_{62} = 5.14, $P = 3.0 \times 10^{-6}$). Body mass did not affect take-off velocity (*SI Appendix*, Fig. S6 and related Supplementary Information).

Reduced Resilin in the Extensor-Tibiae Tendon but not SLPs Correlated with Decreased Take-Off Velocity. *Pro-resilin* RNAi affected the two energy stores in different ways: It reduced

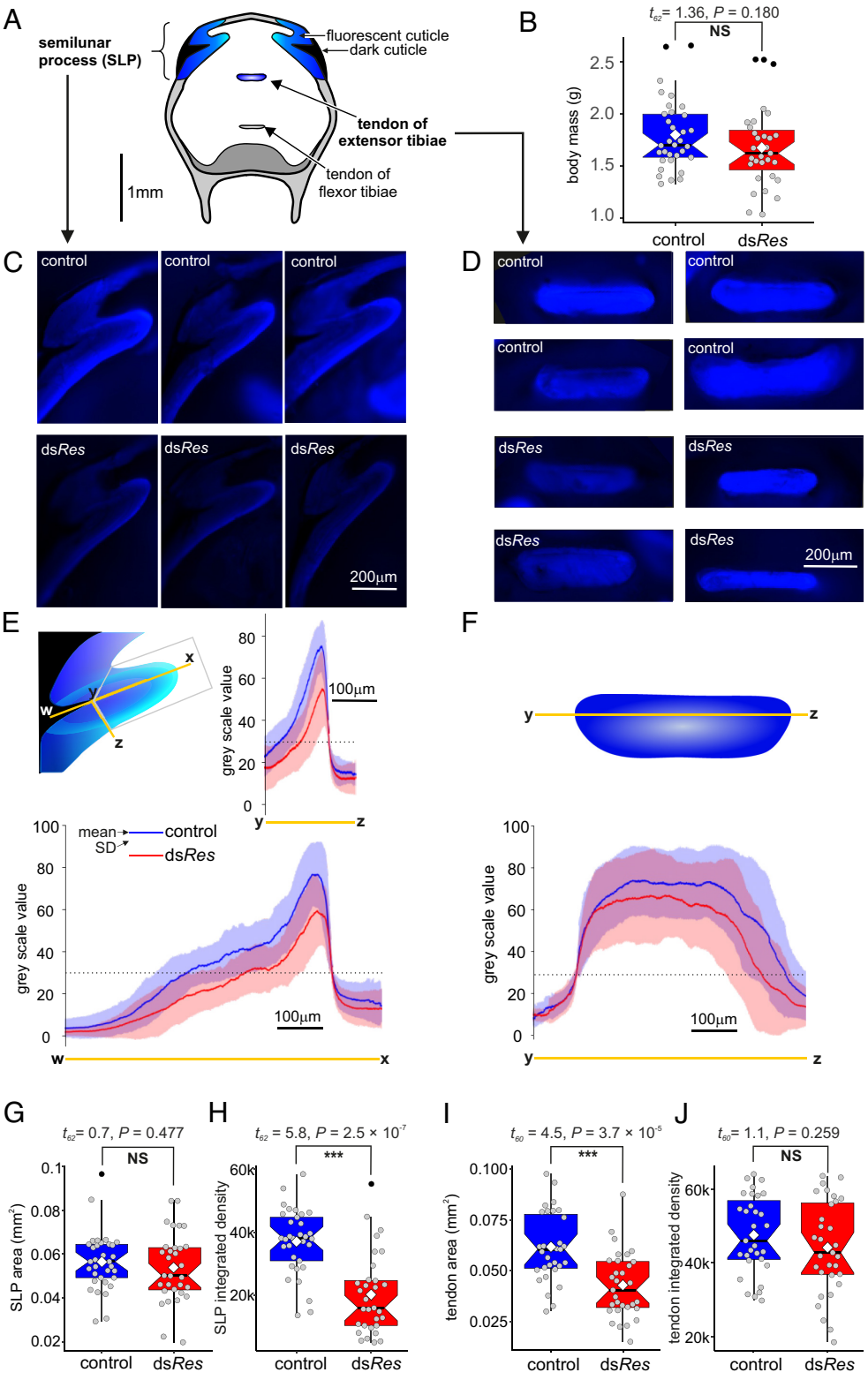

Fig. 2. *Pro-resilin* RNAi reduces resilin in the SLP and extensor tendon. (*A*) Cross-section of the FT joint at the level shown by the red dotted line in Fig. 1*B*. (*B*) Control (blue, *N* = 33) and ds*Res* (red, *N* = 31) locusts did not differ significantly in body mass: box plots with individual data points indicated in gray, outliers in black. Means = white diamonds; medians = black horizontal bars; boxes = interquartile range; whiskers = 10th and 90th decile. (*C*) UV-DAPI fluorescent photomicrographs in same orientation as (*A*) of SLPs in representative control (*Top* row) and ds*Res* locusts (*Bottom* row). Resilin is indicated by the bright blue fluorescence. (*D*) UV-DAPI fluorescent photomicrographs of transverse sections of the extensor-tibiae tendon in representative control (*Top* two rows) and ds*Res* locusts (*Bottom* two rows). (*E*) Mean (lines) ± SD (paler bands) gray-scale intensity of resilin fluorescence along two transects across the SLP, as indicated by the yellow lines w–x and y–z in the inset diagram. Control locusts (blue, *N* = 33) showed greater fluorescence across the SLP than ds*Res* locusts (red, *N* = 31). (*F*) Mean ± SD gray-scale intensity of resilin fluorescence along a transect across the extensor tendon, as indicated by the yellow line y–z. The tendon was narrower in ds*Res* (red, *N* = 31) than in control (blue, *N* = 31) locusts, but fluorescence was similar in both treatments. (*G*) Box plots of the area of the SLP as bounded by the gray box in the inset in *E*. There was no significant difference between treatments. (*H*) Box plots of the gray scale intensity of the SLP along the two transects shown in *E*. ds*Res* locusts had a significantly reduced fluorescence compared to controls. (*I*) Box plots showing the cross-sectional area of the extensor tendon. ds*Res* locusts had a significantly reduced tendon area. (*J*) Box plots of the gray scale intensity of fluorescence in the extensor tendon across a 400 µm transect. There was no significant difference between treatments. (All statistical tests are *t* tests; Asterisks indicate significant differences: NS = not significant, ***P < 0.001).

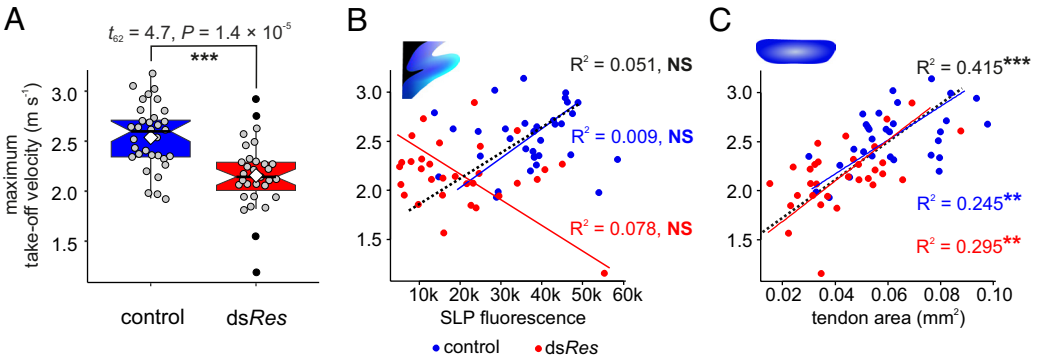

Fig. 3. Relationship between maximum take-off velocity and the amount of resilin in energy stores for jumping. (*A*) Maximum take-off velocity was 15% lower in ds*Res* locusts (red, N = 31) compared to controls (blue, N = 33). Box plots with individual data points indicated in gray, outliers in black; means = white diamond; medians = black horizontal bar; Boxes = interquartile range; whiskers = the 10th and 90th decile; (*t* test; ***P < 0.001). (*B*) The intensity of resilin fluorescence in the SLP plotted against maximum take-off velocity and fitted with standardized major axis regressions: there were no significant correlations (NS) between the integrated density of fluorescence and jumping performance, either within treatments (control blue, P = 0.602; ds*Res* red, P = 0.158) or across all the data (black dotted line, P = 0.071). The low R^2 values suggest minimal explanatory power. (*C*) The cross-sectional area of the extensor-tibiae tendon plotted against maximum take-off velocity and fitted with standardized major axis regressions. Tendon area correlated with take-off velocity, both within treatments (control blue, P = 0.005; ds*Res* red, P = 0.002) and across all the data (black dotted line, P = 1.6 × 10^{-9}; **P < 0.01, ***P < 0.001). Overall, approximately 40% of the variation in jump velocity could be explained by tendon area. The similar intercepts and slopes of control and ds*Res* locusts suggested a lack of other explanatory variables.

resilin fluorescence intensity in the SLP, but did not change its dimensions; and it reduced the cross-sectional area of the extensor-tibiae tendons, but without a reduction in integrated fluorescence intensity. Standardized major axis regression [SMA; (37)] was used to analyze the relationship between these effects and jump velocity. There was no significant correlation between the fluorescence of the SLP and maximum take-off velocity (Fig. 3*B*), either by treatment (R^2 = 0.009, P = 0.602 for controls; R^2 = 0. 078, P = 0.128, for ds*Res* locusts) or across all locusts (R^2 = 0.051, P = 0.071). The low R^2 values suggest minimal explanatory power.

By contrast, extensor tendon area correlated significantly with maximum take-off velocity with substantially stronger R^2 values, both at the level of the individual treatments (Fig. 3*C*; R^2 = 0.246, P = 0.005 for controls; R^2 = 0. 295, P = 0.002, for ds*Res* locusts) and across all locusts (R^2 = 0.415, P = 1.6 × 10^{-9}). Furthermore, both control and ds*Res* locusts fell along a single fitted line, albeit in different regions (Fig. 3*C*; no difference in slope elevation between treatments, $Wald_1$ = 0.105, P = 0.745; shared a common gradient, Likelihood $ratio_1$ = 0.657, P = 0.418; occurred in different regions of the common slope, $Wald_1$ = 25.38, P = 4.7 × 10^{-8}). Similar results were obtained using mean jump velocity (*SI Appendix*, Fig. S5 *B* and *C*). Tendon area accounted for over 40% of the variation in take-off velocity; a possible mechanism is outlined in Supplementary Information.

***Pro-Resilin* RNAi Rendered Locusts More Prone to Jump-Related Damage.** To test the hypothesis that resilin protects elastic energy stores from damage, we compared how control and ds*Res* locusts repeatedly withstood high muscular forces during jump preparation. The FT joint was restrained, and direct electrical stimulation was used to activate the extensor-tibiae muscle, in a pattern mimicking the natural firing rate of the Fast Extensor Tibiae motor neuron (FETi; see example in Movie S1) (34, 38). Longer stimulation resulted in greater forces being developed and greater SLP strain (Fig. 4 *A*, *B*, and *E*). When stimulation ceased, the SLP returned to its resting position (Fig. 4*B*). A breakage occurred in four out of fourteen ds*Res* locusts (Fig. 4 *C* and *D* and Movie S1). In these cases, the SLP initially followed the same bending pattern, until it suddenly ceased to respond to any further stimulation and subsequently underwent only minor displacements (approximately 10 µm; pink line in Fig. 4*E*). In three cases, the moment of breakage was recorded (Fig. 4*C*). The tip of the SLP started moving as in control specimens, but then suddenly stopped bending prior to the end of the stimulus and snapped back to near its starting position within one video frame (<8 ms; see example in Movie S1). By contrast to the 29% failure in ds*Res* locusts, none of the 15 control locusts broke (Fig. 4*D*). In undamaged ds*Res* locusts, SLP displacements caused by stimulation were not statistically different from those of control locusts (repeated measures ANOVA: effect of stimulus length $F_{4, 27}$ = 73.18, P = 7.2 × 10^{-15}; effect of treatment, $F_{1, 27}$ = 0.4, P = 0.807; see Supplementary Information).

A final analysis of the effect of *pro-resilin* RNAi was undertaken by measuring the jump distances of locusts encouraged to jump ten times in quick succession. Jump distances declined in both groups, but after 10 jumps, control locusts still jumped 81 ± 14.6 % as far as their first jump, whereas ds*Res* locusts only achieved 55 ± 13.2 % of their initial distance (Fig. 4*G*; jump distances of ds*Res* locusts were significantly shorter than those of control locusts from the 5th jump onward; *t* tests, P < 0.05; *SI Appendix*, Table S2). Furthermore, one of the ds*Res* locusts refused to jump after the 6th attempt, consistent with a breakage having occurred inside the leg.

Discussion

Resilin is ubiquitous in the elastic energy stores of insects, but its precise roles have long remained elusive (24). RNAi reduced resilin deposition in two key jumping-related energy stores of locusts: a 44% reduction in resilin-induced fluorescence in the SLP, and a 31% reduction in the cross-sectional area of the extensor tendon. This had three principal effects: a significant but modest reduction in the maximum take-off velocity; a substantial increase in the occurrence of breakages during sustained muscle contraction; and a decrease in the distances achieved during repeated jumping.

We achieved a 98% knockdown of *pro-resilin* mRNA but the phenotypic reduction of resilin was only 30 to 44%. Disparities between mRNA and protein expression have been widely noted (39, 40) and are an important factor in determining RNAi efficacy (30, 41, 42). The turnover rate of proteins, which may be affected by compensatory plasticity when protein synthesis declines, can strongly affect the extent of phenotypic knockdown (41). Resilin is completely insoluble in all solvents which do not break peptide bonds (24), making direct protein quantification of resilin

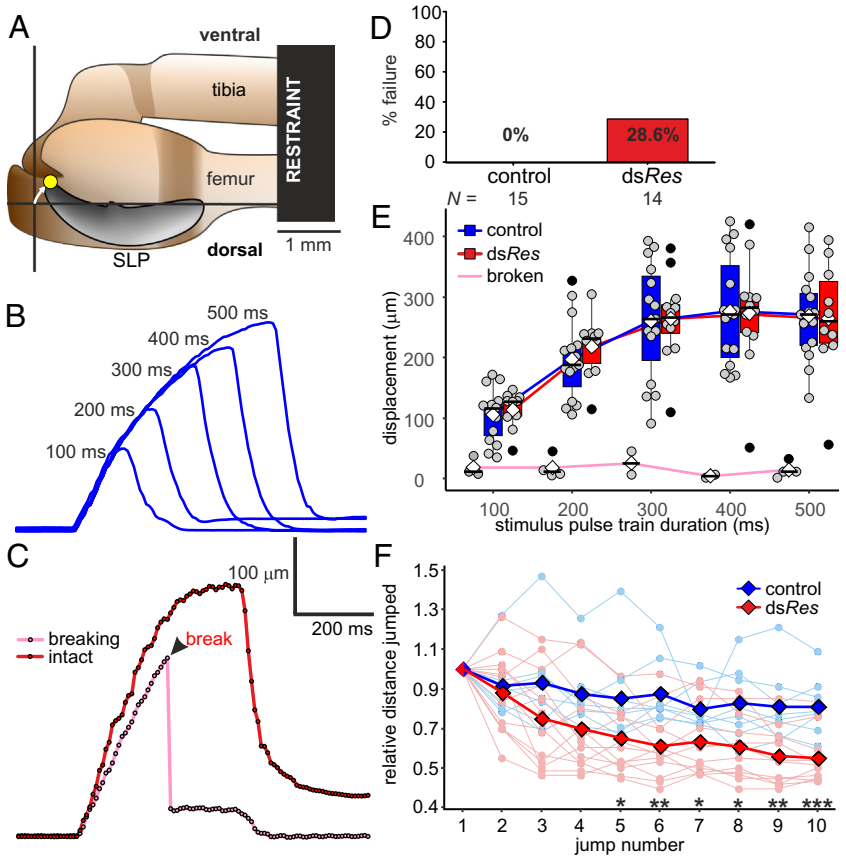

Fig. 4. Forced cocontraction of the extensor-tibiae muscle by trains of electrical pulses at 50 Hz induced breakages in ds*Res* locusts. (*A*) Excursion (white arrow) of the distal tip of the SLP (yellow dot) from its resting position during electrical stimulation of the extensor muscle during tibial restraint. (*B*) Excursions and subsequent relaxations for stimulus periods increasing from 100 to 500 ms. (*C*) An example of a breakage occurring in a ds*Res* locust during stimulation (pink) compared to a normal response to the same 400 ms stimulus (red). The scale applies to both *B* and *C*. (*D*) The incidence of breakages in ds*Res* locusts compared to controls. (*E*) SLP excursions in nonbreaking ds*Res* locusts (red) were similar to those of controls (blue). After breakages, only small excursions were observed (approximately 10 µm; pink). Black horizontal bars = medians; white diamonds = means. (*F*) ds*Res* locusts (red, *N* = 13) experienced a greater decrease in mean performance on being made to jump repeatedly in rapid succession compared to controls (blue, *N* = 8). Data normalized to the first distance jumped for each animal. Jump distances were significantly different from the 5th jump onward; *t* tests, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

extremely difficult. We were therefore only able to measure mature resilin abundance indirectly, through comparative fluorescence of its constituent dityrosine bonds, and our analysis assumes that the proportion of dityrosine cross-linking is similar in control and ds*Res* locusts (43). That the fluorescence of the extensor tendon was unaffected by the RNAi treatment (Fig. 2*J*) suggests that a potential off-target effect that reduced the prevalence of dityrosine, rather than reducing the amount of resilin, was not a substantial issue. Previous work, using a GFP-tagged *pro-resilin*, found a high degree of congruence between fluorescence signatures of dityrosine and GFP (35). The locust genome may contain more than one *pro-resilin* gene and, if so, this might have limited the extent of the possible knockdown. Regardless of the level of knockdown achieved, the phenotypic effects were pronounced.

The modest decrease in take-off velocity suggests that resilin may not be the main elastic energy store and supports the contention that the springs of jumping insects mostly consist of stiffer materials (10, 11, 20). We found no correlation between the intensity of resilin fluorescence in the SLP and jump velocity; no difference in the indentation modulus of the SLP between control and ds*Res* locusts, and SLP dimensions remained unaltered by the RNAi treatment, suggesting that the elastic properties of the SLP remained largely unaffected by the reduction in resilin. Instead, the decrease in jumping performance correlated with the reduced cross-sectional area of the extensor-tibiae tendon in ds*Res* locusts (see Supplementary Information). In principle, an equal muscle force could stretch thinner tendons further to allow the storage of more elastic energy. The spring properties of tendons, however, are likely matched to the length-tension properties of their attached muscles, and changing tendon stiffness without inducing a corresponding change in muscle produces a functional mismatch, reducing the ability to convert muscle work capacity into elastic strain energy (44). Furthermore, it is unclear whether the jumping motor program could respond to the increased tendon compliance in ds*Res* locusts: No proprioceptors directly monitor extensor-tibiae muscle force or the strain in its tendon (45–48), and the jumping motor pattern can be evoked without requiring any sensory feedback (49). Jump orientation is unaffected by one hind leg releasing before the other, or even by one of the hind legs being missing (50, 51): Even if the RNAi treatment differentially affected the two hind legs, this will not have led to a systematic bias in jumping direction with consequent erroneous measurements of take-off velocity.

The high incidence of breakages in ds*Res* locusts suggests that the primary function of resilin may be protective (10, 20). The safety factor of locust jumping is estimated to be just 20% (8), so that even small changes could substantially increase the occurrence of catastrophic failures. We observed no failures in control locusts and were therefore unable to establish a natural failure rate, but it is estimated that 1 in 500 jumps results in a breakage in wild-type locusts (8). The electrical pulses were a strong stimulation regime, which may have contributed to the high incidence of breakages in ds*Res* locusts that had already been made to jump. Repeated stresses, and the accumulated fatigue it produces, can break

tendons using far weaker forces than are needed to break them from a single pull (52).

How could resilin exert its protective effect? The SLP and extensor-tibiae tendon are fibrous composite materials, where proteins, including resilin, form a matrix in which fibrils of chitin are intermeshed. In structures that routinely only undergo small strains, such as skeletal cuticle or bone, the fibrils (chitin or collagen) are less stiff than the matrix (polyphenol-tanned protein or hydroxyapatite). Here, composite construction increases the toughness of materials because the fibrils hinder the formation and propagation of cracks (53, 54). Even so, bone, for example, will mechanically fail at 3% strain (55). In contrast, a distinctive feature of biological springs is that they undergo large repetitive strains during normal function—the SLP strains 8% and the extensor tendon 3.2% (8)—and in these structures the chitin fibrils are actually stiffer than the resilin-containing matrix (25, 56). Some empirical and theoretical studies have analyzed how elastic matrices can impart toughness to more brittle fibrous elements that would otherwise break under high strains (57–59). It is possible that the elastic resilin-containing matrix allows chitin fibers to microbuckle (57, 59) instead of succumbing to large compressive strains and fracturing, temporarily absorbing and distributing imposed energy across a wider volume, since stretched resilin has reduced entropy compared to its relaxed state (60).

A more subtle effect of the protective role of resilin may be reflected by the reduced distance over repeated jumping in ds*Res* locusts. This cannot have been because of substantial breakages, since the insects remained capable of jumping. It is possible that microdamage in ds*Res* locusts reduced elasticity but did not preclude energy storage per se. Alternatively, perhaps resilin has the ability to rapidly restore capacity in the springs, which was compromised in ds*Res* locusts. Spirally bound fibrous structures, such as chitin in the SLP, may undergo plastic deformation due to creep when subjected to repeated bending forces (53), which resilin may counteract by pulling loosening fibers together. The combination of resilin and stiffer cuticle may work similarly to a composite archery bow, which have three advantages over simple bows: They lose less energy to vibration; their performance deteriorates less with repeated use; and they can be kept strung for longer periods (61). In jumping locusts, similar properties would allow a more efficient transfer of elastic energy; an ability to jump repeatedly with little loss of performance (as we observed); and the capability to keep their FT joints fully loaded for extended periods and thus ready to jump, without tension creeping.

Theoretical analysis of energy stored in deformation generally assume a homogeneous spring composition and small strains (<2%), particularly in bending (62–64). The SLP and extensor-tibiae tendon fulfill neither of these criteria. Their large strains violate the linear kinematic and geometric assumptions underlying such analyses. Furthermore, composite materials are challenging to model because it is often unknown how strain will affect their constituent elements in interaction with each other (54), meaning that these structures can only be analyzed empirically. The chitin-binding site on resilin (65) appears to be critical to its normal function and key to achieving the right combination of stiffness, toughness, and protective function. For example, wing posture in *Drosophila* expressing resilin lacking only a functioning chitin-binding domain was phenotypically indistinguishable from that of *Drosophila* completely lacking resilin (35, 66). Conversely, recombinant resilin expressed and polymerized in the absence of chitin is very soft with a modulus of just 2.5 kPa (21).

We have provided evidence that the primary role of resilin is to prevent damage rather than to store energy. Questions remain as to how the structure of mature resilin, and its interaction with the stiffer energy-storing elements of catapult mechanisms such as the SLP, provide this protective function. Further molecular approaches to manipulate the expression and structure of resilin and other cuticular elements of energy-storing springs will provide a powerful tool for investigating the remarkable jumping abilities of insects and other behaviors that depend upon the storage of elastic energy.

Materials and Methods

Desert locusts (*S. gregaria*, Forskål 1775) were taken from a long-established gregarious culture at KU Leuven and used for jumping experiments 12–14 d after molting. A *pro-resilin* transcript sequence was identified from an *S. gregaria* transcriptome [PRJNA752111; (67)] and genome (68), using the protein sequence of Resilin isoform A from the Cat Flea [*Ctenocephalides felis* (22)]. Locusts were microinjected in the hemocoel with double-stranded RNA against either *pro-resilin* (ds*Res*) or *green fluorescent protein* (ds*GFP*, controls) in locust saline (69). All insects were injected nine times to ensure a prolonged and consistent mRNA knockdown, in line with previously established protocols (70, 71): late 4th instar (390 ng in 6 μL); the day of molting to the 5th instar, and after 2 and 5 d (520 ng in 8 μL); and on the day of molting to adulthood, and then 3, 6, 9, and 12 d later (650 ng in 10 μL). We measured the elastic modulus of the SLP in ds*Res* and control locusts using nanoindentation ($N = 7$ of each). See Supplementary Information for further details.

Jump Kinematics. High-speed video (1000 s^{-1} frame rate, 0.2 ms exposure, 1024 × 1024 pixel resolution) was used to record jumping performance of ds*Res* ($N = 31$) and control ($N = 33$) locusts at 28 to 34 °C. Preweighed locusts (to nearest mg) were placed on a platform topped with 12-mm-thick closed cell foam (Plastazote, Watkins and Doncaster, Leominster, UK) and encouraged to jump parallel to the image plane of a Photron Fastcam SA3 camera (Photron Europe, High Wycombe, Bucks, UK) by gently poking the abdomen with a fine paintbrush. Jumps that deviated by >30° from the camera plane were discarded. Kinematic data were extracted using Tracker software (http://physlets.org/tracker/), which automatically tracked a dot of white paint on the thorax near the center of gravity. The time to take-off was defined as the period from the first movement of the hind tibiae to the loss of ground contact. Five jumps were recorded per individual; mean and maximum take-off velocity across all trials per individual were used for data analysis.

Histology/Anatomy. The amount of resilin in one SLP and the extensor tendon were determined for one hind leg from all locusts used in the jumping experiments. Transverse sections were cut through the SLPs at their midpoint with a razor blade (Figs. 1*B* and 2*A*), immersed in locust saline (69), and viewed on an Olympus BX51WI microscope (Olympus, London, UK) under UV light conditioned by a DAPI filter set (DAPI-5060B, Semrock, Rochester, NY) with a sharp-edged (1% transmission limits) band from 350 to 407 nm. The resulting blue fluorescence emission was collected in a similarly sharp-edged band from 413 to 483 nm through a dichroic beam splitter. Images were captured with a Micropublisher 5.0 digital camera (Q Imaging, Marlow, Bucks, UK). The total integrated density of resilin-induced fluorescence in the SLP above a cutoff gray scale value of 30 (Fig. 2*D*) was measured using ImageJ Fiji across two transects, one from the apex of the internal ridge of the SLP, the other near orthogonal to this as indicated in Fig. 2*E*. The transverse areas of the extensor tendon and the SLP were measured by applying a threshold to gray-scale photographic images that extended to cover the tendon and then measuring the area using the analyze particles function in ImageJ Fiji [Fig. 2*E*; (72)].

Electrical Stimulation of the Extensor-Tibiae Muscle. ds*Res* ($N = 14$) and control ($N = 15$) locusts were immobilized ventral side up in plasticine, with a hind tibia locked in a fully flexed position with further plasticine. Two stainless steel wires (50 μm diameter) were inserted 10 mm apart in the hind femur and connected to a Master-8 pulse stimulator (AMPI, Jerusalem, Israel). The extensor muscle was made to contract by stimulating with 2 to 5 V, 1 ms pulses delivered at 50 Hz, simulating FETi (34, 38), delivered for 100, 200, 300, 400, and 500 ms in a randomized order. The SLP of the stimulated leg was videoed with the Photron Fastcam SA3 camera (125 s^{-1} frame rate, 1 ms exposure) and the displacement of a small dot of white paint on its distal tip measured with Tracker.

Repeated Jumping. ds*Res* ($N = 12$) and control ($N = 8$) locusts were individually placed on the floor and encouraged to jump repeatedly in quick succession. Wings were fixed with a small strip of tape to prevent flight and the room maintained at 32 ± 1 °C. For each locust, take-off and landing positions were recorded for ten rapid successive jumps.

Statistics. Standardized major axis (SMA) regressions, and associated tests of slope significance and shifts between treatments were produced using the package SMATR in R (37, 73). SMA accounts for error in both axes and allowed us to test for significant shifts between control and ds*Res* locusts along a common slope (37). All other tests, principally ANOVA, Mann–Witney, and *t* tests as specified in the text, were performed using NCSS version 10 (Kaysville, Utah), after assessing the homogeneity of variance of the data and transforming accordingly.

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information.

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1. R. M. Alexander, "Functions of elastomeric proteins in animals" in *Elastomeric Protein Structures, Biomechanical Properties, and Biological Roles* (Cambridge University Press, 2003), pp. 1–16.
2. R. M. Alexander, Leg design and jumping technique for humans, other vertebrates and insects. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **347**, 235–248 (1995).
3. M. Burrows, G. P. Sutton, The effect of leg length on jumping performance of short- and long-legged leafhopper insects. *J. Exp. Biol.* **211**, 1317–1325 (2008).
4. F. E. Zajac, Muscle and tendon: Properties, models, scaling, and application to biomechanics and motor. *Crit. Rev. Biomed. Eng.* **17**, 359 (1989).
5. M. F. Bobbert, Effects of isometric scaling on vertical jumping performance. *PLoS ONE* **8**, e71209 (2013).
6. H. C. Bennet-Clark, "Jumping in orthoptera" in *Biology of Grasshoppers* (Wiley Interscience, 1990), pp. 173–204.
7. M. Ilton *et al.*, The principles of cascading power limits in small, fast biological and engineered systems. *Science* **360**, eaao1082 (2018).
8. H. C. Bennet-Clark, The energetics of the jump of the locust *Schistocerca gregaria*. *J. Exp. Biol.* **63**, 53–83 (1975).
9. W. Gronenberg, Fast actions in small animals: Springs and click mechanisms. *J. Comp. Physiol. A* **178**, 727–734 (1996).
10. M. Burrows, G. P. Sutton, Locusts use a composite of resilin and hard cuticle as an energy store for jumping and kicking. *J. Exp. Biol.* **215**, 3501–3512 (2012).
11. S. N. Patek, D. M. Dudek, M. V. Rosario, From bouncy legs to poisoned arrows: Elastic movements in invertebrates. *J. Exp. Biol.* **214**, 1973–1980 (2011).
12. H. C. Bennet-Clark, E. C. A. Lucey, The jump of the flea: A study of the energetics and a model of the mechanism. *J. Exp. Biol.* **47**, 59–76 (1967).
13. M. Rothschild, Y. Schlein, K. Parker, S. Sternberg, Jump of the Oriental Rat Flea *Xenopsylla cheopis* (Roths.). *Nature* **239**, 45–48 (1972).
14. M. Burrows, Froghopper insects leap to new heights. *Nature* **424**, 509–509 (2003).
15. M. Burrows, G. Sutton, Interacting gears synchronize propulsive leg movements in a jumping insect. *Science* **341**, 1254–1256 (2013).
16. I. Siwanowicz, M. Burrows, Three dimensional reconstruction of energy stores for jumping in planthoppers and froghoppers from confocal laser scanning microscopy. *eLife* **6**, e23824 (2017).
17. R. H. J. Brown, Mechanism of locust jumping. *Nature* **214**, 939 (1967).
18. W. J. Heitler, M. Burrows, The locust jump. I. The motor programme. *J. Exp. Biol.* **66**, 203–219 (1977).
19. S. M. Rogers *et al.*, Increased muscular volume and cuticular specialisations enhance jump velocity in solitarious compared with gregarious desert locusts, *Schistocerca gregaria*. *J. Exp. Biol.* **219**, 635–648 (2016).
20. M. Burrows, S. R. Shaw, G. P. Sutton, Resilin and chitinous cuticle form a composite structure for energy storage in jumping by froghopper insects. *BMC Biol.* **6**, 41 (2008).
21. C. M. Elvin *et al.*, Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437**, 999–1002 (2005).
22. R. E. Lyons *et al.*, Molecular and functional characterisation of resilin across three insect orders. *Insect Biochem. Mol. Biol.* **41**, 881–890 (2011).
23. G. Qin, X. Hu, P. Cebe, D. L. Kaplan, Mechanism of resilin elasticity. *Nat. Commun.* **3**, 1003 (2012).
24. T. Weis-Fogh, A rubber-like protein in insect cuticle. *J. Exp. Biol.* **37**, 889–907 (1960).
25. S. O. Andersen, T. Weis-Fogh, Resilin. A rubberlike protein in arthropod cuticle. *Adv. Insect Physiol.* **2**, 1–65 (1964).
26. M. Jensen, T. Weis-Fogh, Biology and physics of locust flight. V. Strength and elasticity of locust cuticle. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **245**, 137–169 (1962).
27. M. Rothschild, J. Schlein, J. W. S. Pringle, The jumping mechanism of *Xenopsylla cheopis* I. Exoskeletal structures and musculature. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **271**, 457–490 (1975).
28. M. Rothschild, J. Schlein, K. Parker, C. Neville, S. Sternberg, The jumping mechanism of *Xenopsylla cheopis* III. Execution of the jump and activity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **271**, 499–515 (1975).
29. D. A. Cullen, RNAi unravels the biology of the hemimetabolous and ametabolous insects. *Adv. Insect Physiol.* **42**, 37–72 (2012).
30. N. Wynant, H. Verlinden, B. Breugelmans, G. Simonet, J. Vanden Broeck, Tissue-dependence and sensitivity of the systemic RNA interference response in the desert locust, *Schistocerca gregaria*. *Insect Biochem. Mol. Biol.* **42**, 911–917 (2012).
31. W. J. Heitler, The locust jump: Specialisations of the metathoracic femoral-tibial joint. *J. Comp. Physiol.* **89**, 93–104 (1974).
32. W. J. Heitler, The locust jump. III. Structural specialisations of the metathoracic tibiae. *J. Exp. Biol.* **67**, 29–36 (1977).
33. M. Burrows, Development and deposition of resilin in energy stores for locust jumping. *J. Exp. Biol.* **219**, 2449–2457 (2016).
34. M. Burrows, G. Morris, The kinematics and neural control of high-speed kicking movements in the locust. *J. Exp. Biol.* **204**, 3471–3481 (2001).
35. S. Lerch *et al.*, Resilin matrix distribution, variability and function in *Drosophila*. *BMC Biol.* **18**, 195 (2020).
36. E. Appel, J. Michels, S. N. Gorb, Resilin in insect flight systems. *Adv. Funct. Mater.* **34**, 2215162 (2024).
37. D. I. Warton, R. A. Duursma, D. S. Falster, S. Taskinen, smatr 3– an R package for estimation and inference about allometric lines. *Methods Ecol. Evol.* **3**, 257–259 (2012).
38. M. Burrows, Motor patterns during kicking movements in the locust. *J. Comp. Physiol. A* **176**, 289–305 (1995).
39. T. Maier, M. Güell, L. Serrano, Correlation of mRNA and protein in complex biological samples. *FEBS Lett.* **583**, 3966–3973 (2009).
40. C. Vogel, E. M. Marcotte, Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **13**, 227–232 (2012).
41. J. G. Scott *et al.*, Towards the elements of successful insect RNAi. *J. Insect Physiol.* **59**, 1212–1221 (2013).
42. N. Thakur, J. K. Mundey, S. K. Upadhyay, "RNAi–Implications in entomological research and pest control" in *RNA Interference*, I. Y. Abdurakhmonov, Ed. (InTech, 2016).
43. S. O. Andersen, Regional differences in degree of resilin cross-linking in the desert locust, *Schistocerca gregaria*. *Insect Biochem. Mol. Biol.* **34**, 459–466 (2004).
44. M. V. Rosario, G. P. Sutton, S. N. Patek, G. S. Sawicki, Muscle–spring dynamics in time-limited, elastic movements. *Proc. R. Soc. B Biol. Sci.* **283**, 20161561 (2016).
45. W. J. Heitler, M. Burrows, The locust jump. II. Neural circuits of the motor programme. *J. Exp. Biol.* **66**, 221–241 (1977).
46. M. Burrows, H. J. Pflüger, Positive feedback loops from proprioceptors involved in leg movements of the locust. *J. Comp. Physiol. A* **163**, 425–440 (1988).
47. K. Sasaki, M. Burrows, Proprioceptors monitoring forces in a locust hind leg during kicking form negative feedback loops with flexor tibiae motor neurons. *J. Exp. Biol.* **206**, 759–769 (2003).
48. M. Burrows, *The Neurobiology of an Insect Brain* (Oxford University Press, 1996).
49. W. J. Heitler, Quasi-reversible photo-axotomy used to investigate the role of extensor muscle tension in controlling the kick motor programme of grasshoppers. *Eur. J. Neurosci.* **7**, 981–992 (1995).
50. G. P. Sutton, M. Burrows, The mechanics of elevation control in locust jumping. *J. Comp. Physiol. A* **194**, 557–563 (2008).
51. R. D. Santer, Y. Yamawaki, F. C. Rind, P. J. Simmons, Motor activity and trajectory control during escape jumping in the locust *Locusta migratoria*. *J. Comp. Physiol. A* **191**, 965–975 (2005).
52. X. T. Wang, R. F. Ker, R. M. Alexander, Fatigue rupture of wallaby tail tendons. *J. Exp. Biol.* **198**, 847–852 (1995).
53. S. A. Wainwright, W. D. Biggs, J. D. Currey, J. M. Gosline, *Mechanical Design in Organisms* (John Wiley & Sons, 1976).
54. M. M. Shokrieh, S. M. Ghoreishi, M. Esmkhani, "Toughening mechanisms of nanoparticle-reinforced polymers" in *Toughening Mechanisms in Composite Materials*, (Elsevier, 2015), pp. 295–320.
55. C.-Y. Lin, J.-H. Kang, Mechanical properties of compact bone defined by the stress-strain curve measured using uniaxial tensile test: A concise review and practical guide. *Materials* **14**, 4224 (2021).
56. J. F. V. Vincent, U. G. K. Wegst, Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.* **33**, 187–199 (2004).
57. T. W. Murphey, W. Francis, B. Davis, J. M. Mejia-Ariza, "High strain composites" in *2nd AIAA Spacecraft Structures Conference* (American Institute of Aeronautics and Astronautics, 2015).
58. F. López Jiménez, S. Pellegrino, Folding of fiber composites with a hyperelastic matrix. *Int. J. Solids Struct.* **49**, 395–407 (2012).
59. W. Francis, M. Lake, J. S. Mayes, "A review of classical fiber microbuckling analytical solutions for use with elastic memory composites" in *47th AIAA/ASME/ASCE/AHS/ASC Structures in Structural Dynamics, and Materials Conference* (American Institute of Aeronautics and Astronautics, 2006).
60. T. Weis-Fogh, Thermodynamic properties of resilin, a rubber-like protein. *J. Mol. Biol.* **3**, 520–531 (1961).

61. R. Miller, E. McEwan, C. Bergman, Experimental approaches to ancient near eastern archery. *World Archaeol.* **18**, 178–195 (1986).
62. E. P. Popov, *Engineering Mechanics of Solids* (Prentice-Hall, 1990).
63. R. C. Juvinall, K. M. Marshek, "Chapter 12.1" in *Fundamentals of Machine Component Design* (John Wiley & Sons, ed. 5, 2012).
64. A. P. Boresi, R. J. Schmidt, O. M. Sidebottom, "Chapters 2 and 9" in *Advanced Mechanics of Materials* (John Wiley & Sons, ed. 5, 1993).
65. J. E. Rebers, J. H. Willis, A conserved domain in arthropod cuticular proteins binds chitin. *Insect Biochem. Mol. Biol.* **31**, 1083–1093 (2001).
66. S. Lerch, Y. Yang, J. Flaven-Pouchon, N. Gehring, B. Moussian, *Resilin* is needed for wing posture in *Drosophila suzukii*. *Arch. Insect Biochem. Physiol.* **111**, e21913 (2022).
67. L. Ragionieri *et al.*, *Schistocerca* neuropeptides–An update. *J. Insect Physiol.* **136**, 104326 (2022).
68. H. Verlinden *et al.*, First draft genome assembly of the desert locust, *Schistocerca gregaria*. *F1000Research* **9**, 775 (2020).
69. P. N. R. Usherwood, H. Grundfest, Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497–518 (1965).
70. C. Lenaerts *et al.*, The ecdysis triggering hormone system is essential for successful moulting of a major hemimetabolous pest insect, *Schistocerca gregaria*. *Sci. Rep.* **7**, 46502 (2017).
71. J. Lv *et al.*, RNAi-mediated silencing of the neverland gene inhibits molting in the migratory locust, *Locusta migratoria*. *Pestic. Biochem. Physiol.* **200**, 105845 (2024).
72. J. Schindelin *et al.*, Fiji: An open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).
73. R Core Team, *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, 2018).