

Resilin in Dragonfly and Damselfly Wings and Its Implications for Wing Flexibility

Seth Donoughe,¹ James D. Crall,^{2*} Rachel A. Merz,³ and Stacey A. Combes²

¹*Department of Cell and Developmental Biology, University of Pennsylvania Medical School, Philadelphia, Pennsylvania*

²*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts*

³*Department of Biology, Swarthmore College, Swarthmore, Pennsylvania*

ABSTRACT Although there is mounting evidence that passive mechanical dynamics of insect wings play an integral role in insect flight, our understanding of the structural details underlying insect wing flexibility remains incomplete. Here, we use comparative morphological and mechanical techniques to illuminate the function and diversity of two mechanisms within Odonata wings presumed to affect dynamic wing deformations: flexible resilin vein-joints and cuticular spikes. Mechanical tests show that joints with more resilin have lower rotational stiffness and deform more in response to a load applied to an intact wing. Morphological studies of 12 species of Odonata reveal that resilin joints and cuticular spikes are widespread taxonomically, yet both traits display a striking degree of morphological and functional diversity that follows taxonomically distinct patterns. Interestingly, damselfly wings (suborder Zygoptera) are mainly characterized by vein-joints that are double-sided (containing resilin both dorsally and ventrally), whereas dragonfly wings (suborder Epiprocta) are largely characterized by single-sided vein-joints (containing resilin either ventrally or dorsally, but not both). The functional significance and diversity of resilin joints and cuticular spikes could yield insight into the evolutionary relationship between form and function of wings, as well as revealing basic principles of insect wing mechanical design. *J. Morphol.* 272:1409–1421, 2011. © 2011 Wiley Periodicals, Inc.

KEY WORDS: flight; wings; wing flexural stiffness; resilin; Odonata

INTRODUCTION

Animal fliers move using wings that deform substantially during flapping flight (Swartz et al., 1992; Biewener and Dial, 1995; Young et al., 2009). Insect wings differ from those of vertebrates in that they lack internal musculature extending into the aerodynamic surface of the wing. Thus, while birds and bats can actively modulate the form and flexibility of their wings, insects have little active control over wing properties, and most deformations are a product of the passive mechanical properties of the wing (Wootton, 1992) interacting with the inertial and aerodynamic forces it generates while flapping (Daniel

and Combes, 2002). These passive deformations may be an inevitable property of wings that are constructed from flexible, biological materials, yet they often appear to be beneficial (Wootton, 1981).

Flexibility in insect wings may enhance aerodynamic performance. Previous work has revealed a variety of passive deformation mechanisms in insect wings that appear to be aerodynamically useful. For example, the automatic depression of the trailing edge (Wootton, 1991) and twisting of the leading edge under aerodynamic loading (Ennos, 1988) both create camber. The varying position of the nodus in damselfly and dragonfly wings is correlated with the amount of wing twisting in flight, which in turn is related to stroke plane inclination (Wootton and Newman, 2008). Several studies have provided direct evidence that flexible wings that are able to produce camber may generate higher peak lift forces than rigid wings (Mountcastle and Daniel, 2009; Young et al., 2009). By contrast, however, recent work done on model wings has shown that at low and medium angles of attack, aerodynamic performance decreases with increased flexibility (Zhao et al., 2010). Yet, the aerodynamic performance of a flexible model wing can match or even exceed that of rigid wings if rudimentary longitudinal veins are added, probably because the veins promote cambering (Zhao et al., 2010). Thus, the exact role of wing flexibility in aerodynamic performance remains unclear.

Seth Donoughe and James D. Crall should be considered primary authors.

Contract grant sponsor: NSF Expeditions in Computing; Contract grant number: CCF-0926148.; Contract grant sponsors: Swarthmore College (Meinkoth Fund), Walter Kemp's family.

*Correspondence to: James D. Crall, Concord Field Station, 100 Old Causeway Rd, Bedford, MA 01730.
E-mail: jcrall@oeb.harvard.edu

Received 17 November 2010; Revised 26 April 2011;
Accepted 11 May 2011

Published online 13 September 2011 in
Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/jmor.10992

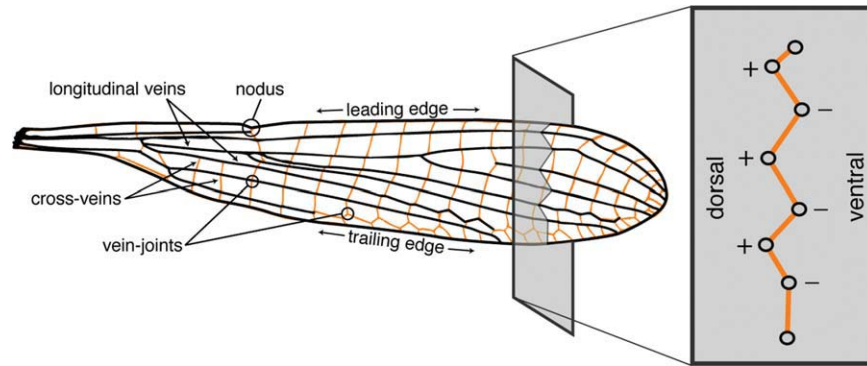


Fig. 1. Damselfly wing with longitudinal veins shown in black and cross-veins in orange. Veins meet to form vein-joints. Inset illustrates the corrugated cross-section of the wing, with alternating “mountain” (+) and “valley” (–) longitudinal veins. The nodus is a specialized, highly conserved vein-joint.

Flexibility in insect wings may also help to protect against permanent wing damage. Newman and Wootton (1986) first suggested that dragonfly wings appear to be adapted for reversible failure in response to excess loads, enabling them to avoid permanent structural damage. Although previous studies have provided conflicting evidence on the general fitness consequences of wing damage (see Cartar, 1992; Kingsolver, 1999; Hedenström et al., 2001), recent work on dragonflies has shown that artificial damage decreases vertical acceleration and impairs prey capture performance (Combes et al., 2010). Thus, at least in dragonflies, there is probably selective pressure against wing damage. Mechanisms allowing for reversible failure of dragonfly wings may therefore represent an important and largely overlooked aspect of wing morphology.

Our goal in this study, especially pressing in light of the renewed interest in the aerodynamic consequences of wing flexibility, was to expand on the existing body of work that investigated the detailed morphological mechanisms underlying overall wing deformations. In particular, we examined the diversity of fine-scale structures associated with wing flexibility within one order of spectacular aerial acrobats: the dragonflies and damselflies (Order: Odonata).

Odonate wings are composed of a thin, translucent bilayer of cuticle (wing membrane) that is reinforced by a meshwork of thickened cuticular veins. Longitudinal veins originate at or close to the base of the wing and run along the length of the wing (Fig. 1, black), whereas smaller cross-veins run perpendicular to and connect longitudinal veins (Fig. 1, orange). Veins meet to form vein-joints (Fig. 1, circled)—the structures that are the focus of this study.

Wootton (1991) reviewed passive mechanisms that enable shape changes within the wings of Odonata and emphasized the importance of understanding the mechanical properties of the junc-

tions between cross-veins and longitudinal veins. Vein-joints that are morphologically specialized for flexibility were first described by Newman (1982) and were later shown to contain resilin (Gorb, 1999), a flexible, rubberlike protein found in many insect locomotory structures (Weis-Fogh, 1960). Resilin functions as a spring in the flea leg (Bennet-Clark and Lucey, 1967) and the frog-hopper pleural arch (Burrows et al., 2008), and is found in the energy-storing tendon at the base of dragonfly and locust wings (Weis-Fogh, 1960). Resilin's high flexibility (Young's modulus around 1 MPa, in contrast to values up to 20 GPa for sclerotized cuticle; Vincent and Wegst, 2004), capacity for energy storage (energy loss of less than 5% over a wide range of frequencies; Jensen and Weis-Fogh, 1962), and near indestructibility under natural conditions (Weis-Fogh, 1960; Anderson, 1963) make it ideally suited for its role in insect locomotion. Resilin has since been found embedded in beetle and earwig wings (Haas et al., 2000a,b, respectively), where it plays a role in wing folding at rest, but to our knowledge, no work other than Gorb (1999) has described resilin structures that may be associated with wing deformations during flight. In this study, we use microscopy and mechanical testing to examine the diversity and function of vein-joints to understand how they contribute to the flexibility and passive deformations of odonate wings.

MATERIALS AND METHODS

Specimen Collection

Specimens were collected from Bedford, MA in the summer of 2009 or Swarthmore, PA in the summers of 2008–2010, and then stored and euthanized in compliance with the regulations of Swarthmore College Institutional Animal Care and Use Committee. Before microscopy, specimens were rehydrated in a humid plastic container incubated at room temperature for at least 12 h. The forewings of each specimen were then removed for morphological investigations.

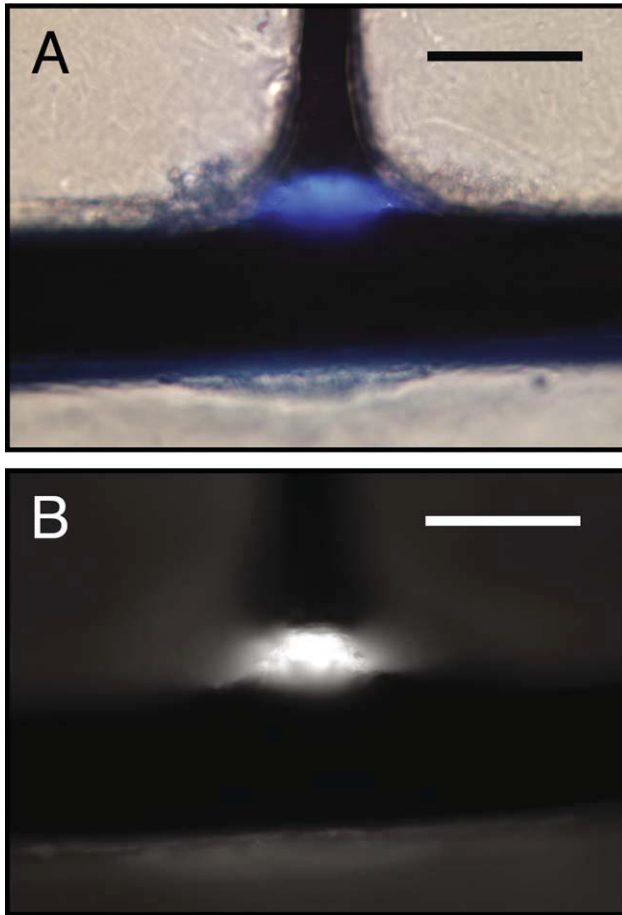


Fig. 2. An example of the confirmation of resilin in the vein-joint. An *A. verticalis* vein-joint imaged with (A) a DAPI filter cube that shows the blue autofluorescence characteristic of resilin and (B) the same joint imaged with a resilin-specific filter cube that reveals fluorescence between 410 and 450 nm. Scale bars represent 20 μm .

Fluorescence Microscopy

For fluorescence microscopy (FM), wings were dry mounted between two cover slips and observed using either a Leica DMRB or a Zeiss Axioplan microscope with a DAPI filter (excitation 340–380 nm, 420 nm longpass emission filter). Wing veins were examined for the presence of resilin, which appears as a deep blue color under UV excitation (see, for instance, Fig. 2A), on both the dorsal and ventral sides. Images were captured with a Zeiss Axioscope digital camera. We provided further evidence for the presence of resilin in a subset of wings by taking monochrome images with a custom chroma filter cube that provides UV excitation at 330–370 nm and allows emission at 410–450 nm, thereby narrowly selecting resilin's known emission peak of ~ 420 nm (Neff et al., 2000; Fig. 2B).

We conducted a detailed, joint-by-joint mapping of resilin on the wings of the damselfly *Ischnura verticalis* and the dragonflies *Sympetrum vicinum* and *Aeshna verticalis*, using a Leica DMRB fluorescent microscope up to a magnification of 40 \times . Veins were identified using the Riek and Kukalova-Peck (1984) wing homology system (as updated by Rehn, 2003). Next, we conducted a wider taxonomic comparison of resilin patterns, this time focusing solely on longitudinal veins. We examined one specimen each of nine additional species, drawing from

several odonate families, including *Aeshna constricta*, *Sympetrum rubicundulum*, *Erythemis simplicicollis*, *Somatochlora tenebrosa*, *Epiheca cynosura*, *Ischnura posita*, *Enallagma divagans*, *Calopteryx augustipennis*, and *Lestes rectangularis*. For this, we used a Zeiss Axioplan fluorescent microscope. The dorsal and ventral sides of each vein were examined separately. A given longitudinal vein was scored as “present” for resilin if any of the vein-joints on that vein showed visible blue fluorescence. Out of 168 examined veins between all included species, 99 were scored as “present” for resilin. Among those veins, 80 had resilin visible in all vein-joints, 12 had resilin visible in 50–99% of vein-joints, and seven had resilin visible in 30–49% of vein-joints.

There were some limitations to our methodology for mapping resilin, as there was subtle variation in the size of resilin patches and whether cuticular projections were present on top of such patches. We found that the visibility of very small patches of resilin in wings was occasionally dependent on the sensitivity of the microscope and how a wing was oriented in its mount. In our own investigation, we found rare discrepancies when the same wing was mapped on the Leica DMRB versus the Zeiss Axioplan, as well as when multiple individuals of the same species were examined (unpublished data). These differences, however, were limited to particular vein-joints within a given vein, and did not extend to the presence or absence of resilin within entire veins. Thus, although there may be joint-specific differences between individuals of the same species, the vein-specific patterns are conserved. Accordingly, we only examined a single specimen from each species while mapping vein-specific patterns.

Scanning Electron Microscopy

After FM imaging, wings were coated with gold–palladium and examined using scanning electron microscopy (SEM) on a Phillips XL20 at an accelerating voltage of 10 kV. The three closely mapped specimens (*I. verticalis*, *S. vicinum*, and *A. verticalis*) were fixed only at the base of the wing, allowing for coating and examination of both dorsal and ventral sides of the wings. We scored each vein-joint for the presence of cuticular spikes in close proximity to a vein-joint. For the remaining specimens in the taxonomic comparison, right and left forewings were each mounted flat on a metal stud, one exposing the dorsal side of the wing and one exposing the ventral side. The number of vein-joints with joint-associated cuticular spikes was tallied along the length of each vein, for both the dorsal and ventral sides, and then presented as a percentage of the total number of vein-joints that were visible along that side of the vein.

Mechanical Tests—Single Joint

Given that resilin is largely found in vein-joints where cross-veins meet longitudinal veins, we sought to assess the role that resilin plays in determining the mobility of individual vein-joints. To measure “mobility,” we asked how much resistance does a single cross-vein face when it pivots around the adjacent longitudinal vein? If resilin promotes flexibility in a vein-joint, we reasoned that a resilin-containing vein-joint would exhibit lower resistance to pivoting than a similar vein-joint that does not contain resilin. We measured this resistance following a protocol adapted from Emlet (1982). An intact forewing from a freshly killed *I. verticalis* was glued on a microscope slide using cyanoacrylate, with the span of the wing parallel to the edge of the slide, leaving approximately half of the wing extending beyond the edge of the slide (Fig. 3A). Iridectomy scissors were used to cut away the wing material surrounding a single vein-joint (Fig. 3B,C), leaving a length of the associated cross-vein (blue) attached to the adjacent longitudinal vein (red).

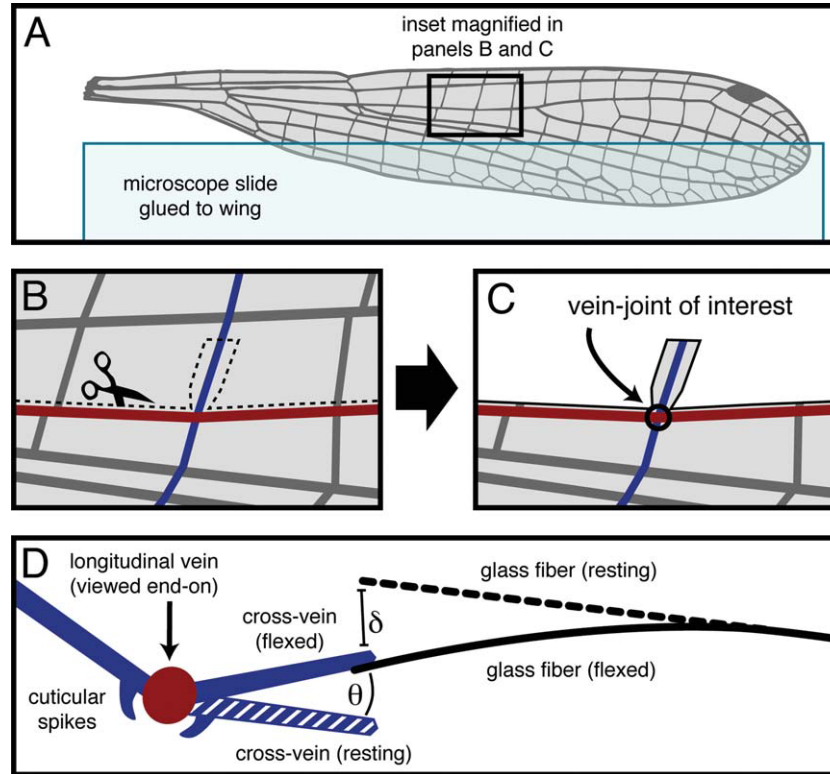


Fig. 3. Procedure for determining the rotational stiffness of individual vein-joints. **A:** A wing from a freshly killed *I. verticalis* was glued to a microscope slide with a portion of the wing extending beyond the edge of the glass. **B:** A vein-joint was chosen where a cross-vein (blue) intersected a longitudinal vein of interest (red). Wing material was dissected away to leave a length of the cross-vein still attached to the vein-joint (**C**). The microscope slide was mounted so that the longitudinal vein could be viewed end-on (**D**). A glass fiber was used to pivot the cross-vein about the vein-joint. A photo was taken with the members flexed and at rest. These were digitally overlaid, allowing us to measure both the extent of rotation of the cross-vein and the displacement of the tip of the glass fiber.

Tests were then performed by pivoting isolated cross-veins with a glass fiber. In general, when one beam is used to apply a force to another, the magnitude of the forces acting on each beam is the same. Additionally, the force acting on a beam can be calculated if its deflection and flexural stiffness (EI) are known (see Eq. 1). Since the EI of the glass fiber was known, we were able to calculate the magnitude of the force of the cross-vein acting on the glass fiber, which was equal to the force acting on the cross-vein.

The glass fiber was brought into contact with the isolated cross-vein, causing it to pivot through 10–15° of rotation about the longitudinal vein. For each measurement, a side-on photo was taken of both the resting and flexed positions of the glass fiber and the cross-vein. These images were overlaid (diagrammed in Fig. 3D), allowing us to measure the deflection of the glass fiber (δ) and the extent of rotation of the cross-vein (θ). The force that the glass fiber exerted on the cross-vein (F) was calculated using the following cantilever beam equation:

$$F = (3 \times EI \times \delta) / (L^3) \quad (1)$$

where EI is the flexural stiffness (2.60×10^{-9} N/m² for the glass fiber), δ is the deflection of the glass fiber at its point of contact with the vein, and L is the length of the glass fiber to its point of contact. For all trials, $\delta/L < 0.05$, as the cantilever beam equation is only valid for relatively small deflections (Faupel, 1964).

As the force on the glass fiber and the force on the cross-vein are equal, we used the force (F) calculated from Eq. 1 to calculate the rotational stiffness in Newton-meters/degree:

$$\text{rotational stiffness (N m/degree)} = F \times d / \theta \quad (2)$$

where F is the force calculated from Eq. 1, d is the distance from the base of the cross-vein to the point of contact with the glass fiber, and θ is the angular deflection of the cross-vein. We report rotational stiffness rather than flexural stiffness because the displacement of the tip of the cross-vein was the result of rotation around the vein base rather than deflection as a cantilever beam.

Mechanical Tests—Whole Wing

The single-joint mechanical tests are useful for making direct measurements of the vein-joints themselves, but they have the drawback of isolating vein-joints from their context within a whole wing. For example, even if an isolated vein-joint is flexible, it could be rendered immobile by surrounding rigid elements, in which case it may not deform when force is applied to the wing. To address this issue, we performed whole-wing bending tests to compare deformations of different joint types in intact wings. For these whole-wing deformation tests, larvae of *Enallagma civile* were ordered from Carolina Biological Supply and reared in the lab on a diet of brine shrimp. After emer-

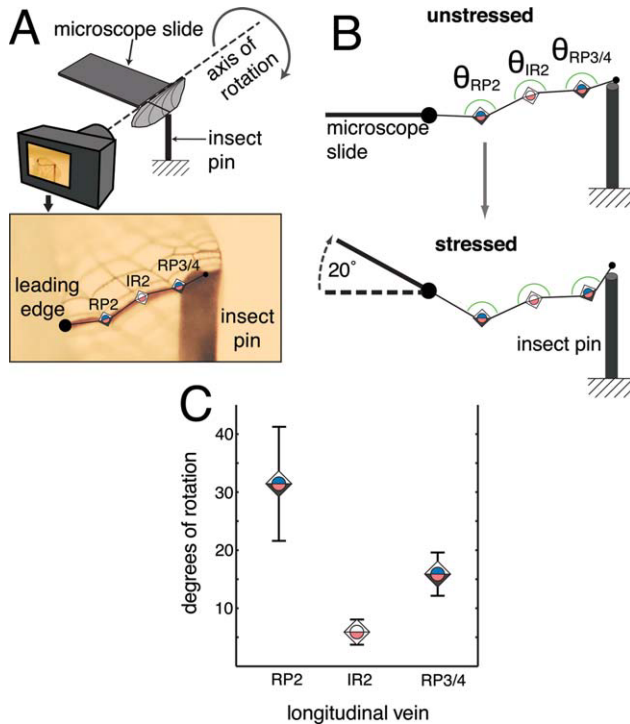


Fig. 4. Apparatus and results for comparing bending of morphologically distinct joints in the damselfly *E. civile*. **A**: Wings were dissected at the base, mounted along the leading edge spar to a microscope slide attached to a micromanipulator, and then (**B**) rotated 20° about the leading edge against a fixed insect pin while measuring angle changes at individual veins RP2, RP3/4, and IR2 (Fig. 9B). **C**: Degree of rotation was calculated as the absolute difference between stressed and unstressed joint angles. Student's *t*-tests were conducted to make pairwise comparisons. Along the two veins containing resilin patches both dorsally and ventrally (RP2 and RP3/4), degrees of rotation were significantly higher than in the vein containing minimal resilin (IR2) [RP2 vs. IR2: $N = 11$, $P \ll 0.05$, IR2 vs. RP3/4: $N = 11$, $P \ll 0.05$]. Degree of rotation was also significantly higher in RP2 than RP3/4 [$N = 11$, $P < 0.05$]. See text for details of procedure.

gence, damselflies were allowed to fly freely in a flight cage for at least 3 h to allow ample time for the sclerotization of wing cuticle (typically 60–90 min in flies, Fraenkel and Hsiao, 1965). For mechanical testing, a damselfly was frozen for 5 min and the right forewing was ablated and brought back to room temperature. The wing was mounted on the edge of a microscope slide along the leading edge using cyanoacrylate and immediately cured using baking soda. A black line was marked with a felt-tipped marker along the chord of the wing, transecting veins RP2, IR2, and RP3/4.

The microscope slide was then mounted in a rotational apparatus, with the axis of rotation established as the leading edge of the wing (Fig. 4A). A camera was mounted with its focal plane perpendicular to the axis of rotation of the wing. The camera was focused on the marked chordwise section of the wing. A fixed insect pin was placed under the wing so as to make contact near the trailing edge. Force was applied with a pin to allow for clear visualization of wing deformation and precise application of force. As insect wings are substantially more flexible across the chord than across the span, we assumed that the majority of deformation was occurring across the chord, but it should be noted this method does not take into account potential deformations across the span of the wing.

Images were captured just before the wing made contact with the insect pin and after the wing had rotated through an angle

of $\sim 20^\circ$ in contact with the fixed insect pin (Fig. 4B). Angles around individual veins (RP2, IR2, and RP3/4) were measured in ImageJ, using adjacent veins (or the glued leading edge in the case of RP2) as reference points, and then compared before and after the application of force to the trailing edge.

RESULTS

Detailed Morphological Survey of Joint Morphology

Initial FM on the wings *I. verticalis*, *S. vicinum*, and *A. verticalis* confirmed the presence of the blue-fluorescing material in vein-joints first described in *Enallagma cyathigerum* by Gorb (1999). A resilin-specific custom filter set provided further evidence that this fluorescent material was resilin (Fig. 2).

Resilin was largely found in joints where cross-veins meet longitudinal veins (Figs. 5C, 6C, and 7C, red and blue semicircles). Consistent with previous findings (Gorb, 1999), most of these resilin patches form a strip parallel to the longitudinal vein, appearing to create a flexible hinge (e.g., Figs. 5D—dorsal, 6E—dorsal, and 7E—ventral). Only in a few rare cases (e.g., Fig. 5B—ventral) do resilin patches cross the longitudinal veins. In all three species, a strong patch of resilin traverses the nodus (e.g., Figs. 5A and 7A), a unique structure presumed to play a role in wing torsion (Wootton et al., 1998). Intriguingly, the presence of resilin often differs between the dorsal and ventral sides of individual joints, with some joints containing only dorsal resilin (e.g., Figs. 6A and 7B), some joints containing only ventral resilin (e.g., Figs. 6B and 7E), and others containing resilin on either both the dorsal and ventral sides of the joint (e.g., Figs. 6E and 7D) or neither side of the joint (e.g., Fig. 5E).

Scanning electron micrographs show that odonate wings also have pointed cuticular protrusions (“spikes”) on the apical surface of wing veins where they meet to form joints (Figs. 5C, 6C, and 7C, black triangles). These joint-associated spikes are almost exclusively located on cross-veins, immediately adjacent to longitudinal veins. Some spikes point directly orthogonally to the wing surface (e.g., Fig. 8A,D), whereas others are asymmetrically shaped, pointing toward the adjacent vein-joint (e.g., Fig. 8B,C). In some cases, spikes are visibly in contact with the longitudinal vein (e.g., Figs. 5D—ventral and 8B), whereas in others there is a small space between the spike and the longitudinal vein (Fig. 8A,C, and D). In the latter case, it is sometimes possible to see the imprint of the spike into the waxy coating on the longitudinal vein, evidence that there is contact between the spike and the longitudinal vein (Fig. 8C,D, arrowheads). As with resilin, a given joint may have spikes on the dorsal side only (e.g., Fig. 5, vein RP1), the ventral side only (e.g., Figs. 5B,D, and

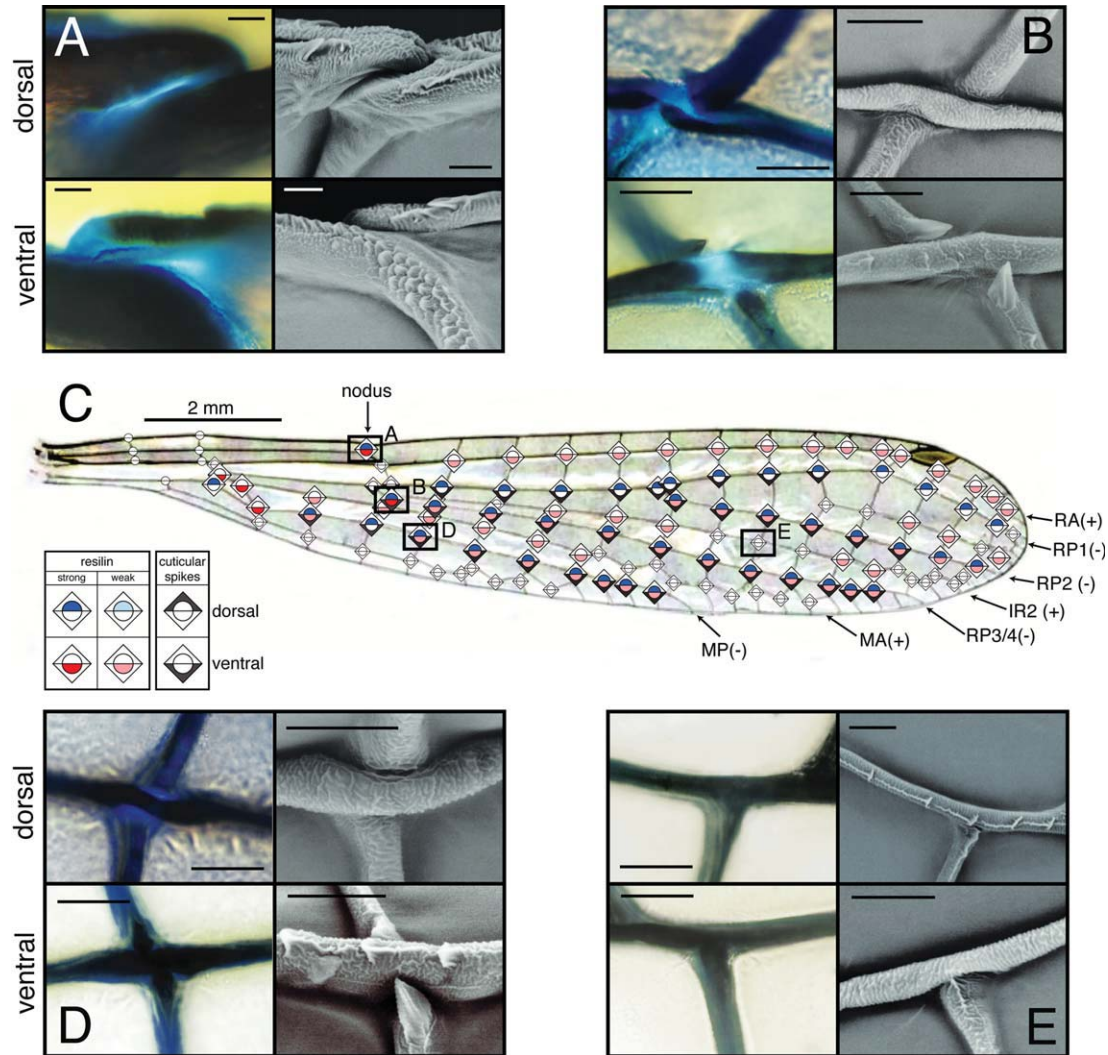


Fig. 5. Dorsal and ventral distribution of resilin pads and cuticular spikes in the vein-joints of a forewing from the damselfly *I. verticalis*. **A,B:** FM (DAPI filter + brightfield light) and SEM images of both the dorsal and ventral sides of selected joints. Scale bars represent 25 μ m. **A:** The nodus has large resilin patches visible from both the dorsal and ventral sides. **B:** One of the rare vein-joints to show resilin crossing the longitudinal (spanwise) vein. Cuticular spikes are present on the ventral side. **C:** Photograph of a wing on which the condition of each joint has been recorded. Major veins are labeled following the system of Riek and Kukalova-Peck (1984; Fig. 9B) and the relief patterns of the veins are designated by “+” (dorsally convex “mountain”) and “-” (dorsally concave “valley”; see Fig. 1). Scale bar represents 2 mm. The inner circles of each symbol represent the presence or absence of resilin: the upper half of each circle indicates resilin dorsally (deep blue if brightly visible, light blue if weak, and white if absent), and the lower half of each circle indicates the presence of resilin ventrally (deep red if strongly present, light red if weak, and white if absent). The diamonds surrounding the inner circle of each diagram indicate whether a cuticular spike is associated with the joint: the upper half of each diamond represents the dorsal side of the joint (dark gray if a cuticular spike present, white if absent) and the lower half represents the ventral side (dark gray if a cuticular spike is present, white if absent). **D,E:** FM (DAPI filter + brightfield light) and SEM images of both the dorsal and ventral sides of selected joints. Scale bars represent 25 μ m. **D:** A joint with strong resilin dorsally and weak resilin ventrally. Spikes are present on the ventral side. **E:** A joint lacking resilin and spikes.

6E), on both the dorsal and ventral sides (e.g., Fig. 6D), or on neither side (e.g., Figs. 5E, 6B, and 7B,D, and E).

Phylogenetic Comparison of Joint Morphology Patterns

Resilin. Our initial joint-by-joint FM and SEM mapping showed that a) resilin patches and cuticular spikes are widespread in odonate wings, b)

resilin patches and spike patterns are fairly consistent along a given longitudinal vein, and c) these longitudinal vein-specific patterns differ between species. Next, we conducted FM and SEM mapping on a wider array of odonates, focusing our attention solely on major longitudinal veins and the nodus. Although the structures of their wings have diverged in many ways, the same set of longitudinal veins is largely conserved among odonates,

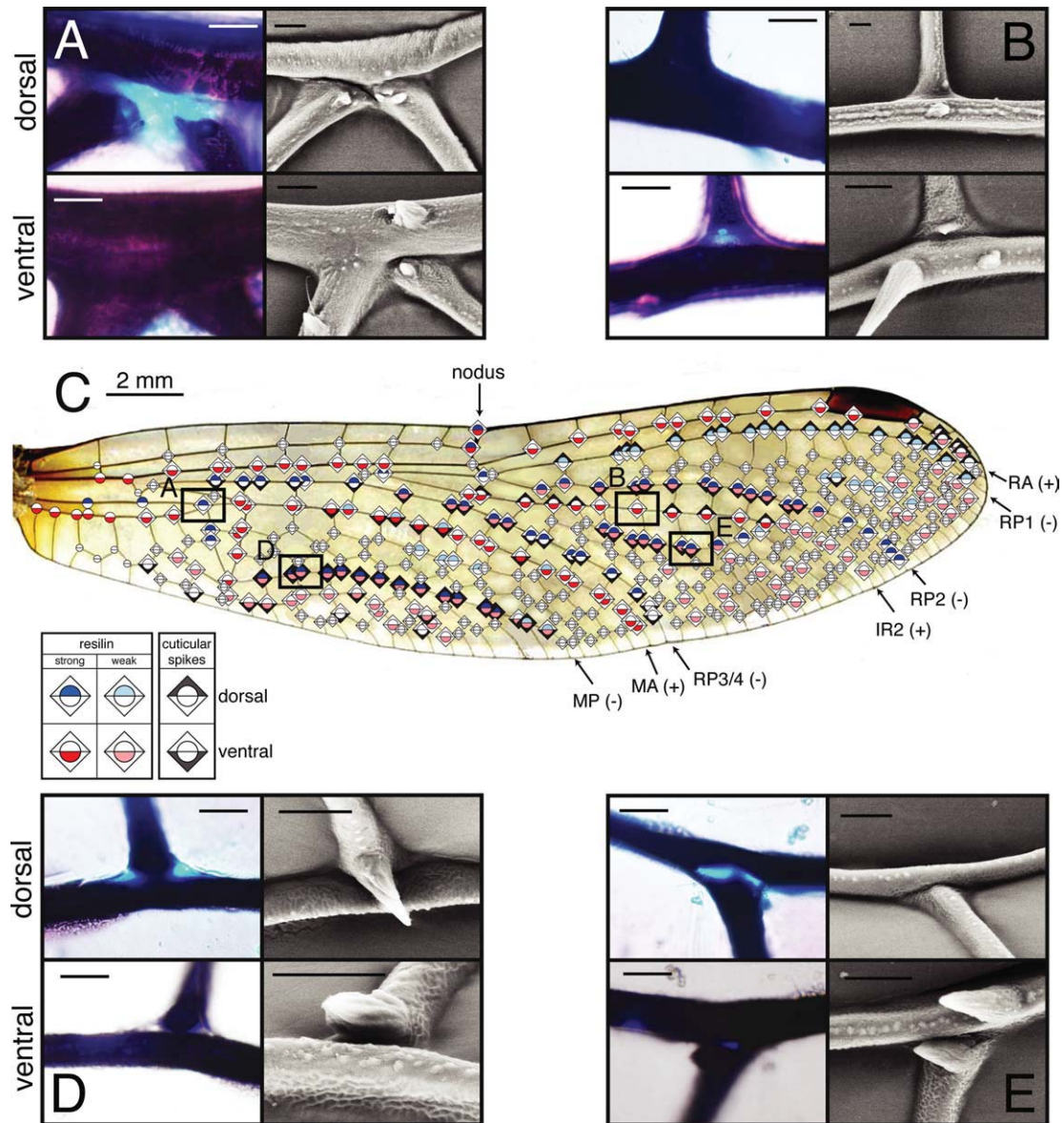


Fig. 6. Dorsal and ventral distribution of resilin pads and cuticular spikes in the vein-joints of a forewing from the dragonfly *S. vicinum*. For further explanation of symbols, see the legend of Figure 5. **A,B**: FM (DAPI filter + brightfield light) and SEM images of both the dorsal and ventral sides of selected joints. Scale bars represent 25 μ m. **A**: Dorsal resilin only. **B**: Ventral resilin only. **C**: Photograph of a wing on which the condition of each joint has been recorded. Scale bar represents 2 mm. **D,E**: FM (DAPI filter + brightfield light) and SEM images of both the dorsal and ventral sides of selected joints. Scale bars represent 25 μ m. **D**: Strong resilin dorsally and weak resilin ventrally. Spikes are present on the dorsal and ventral sides. **E**: Strong resilin dorsally and weak resilin ventrally. Spikes are present on the ventral side.

allowing us to make meaningful morphological comparisons between closely and distantly related groups. The results are summarized in Figure 9A.

Resilin patches are widespread in all odonate species examined. Three trends extend across all groups that we sampled: 1) the nodus is consistently highly enriched with resilin both dorsally and ventrally; 2) longitudinal veins RP2 and RP3/4 always have resilin present dorsally; and 3) the trailing edge vein MP has resilin present

both dorsally and ventrally in all examined species.

For the most part, however, subgroups of odonates were found to display distinct patterns. Within Epiprocta (dragonflies), we observed a remarkable degree of conservation of resilin pattern. With the notable exception of only one vein (MP), resilin tends to be found in the concave, or "valley" (see Fig. 1), side of the longitudinal veins we examined (Fig. 9A, group 1; see Fig. 10A for schematic).

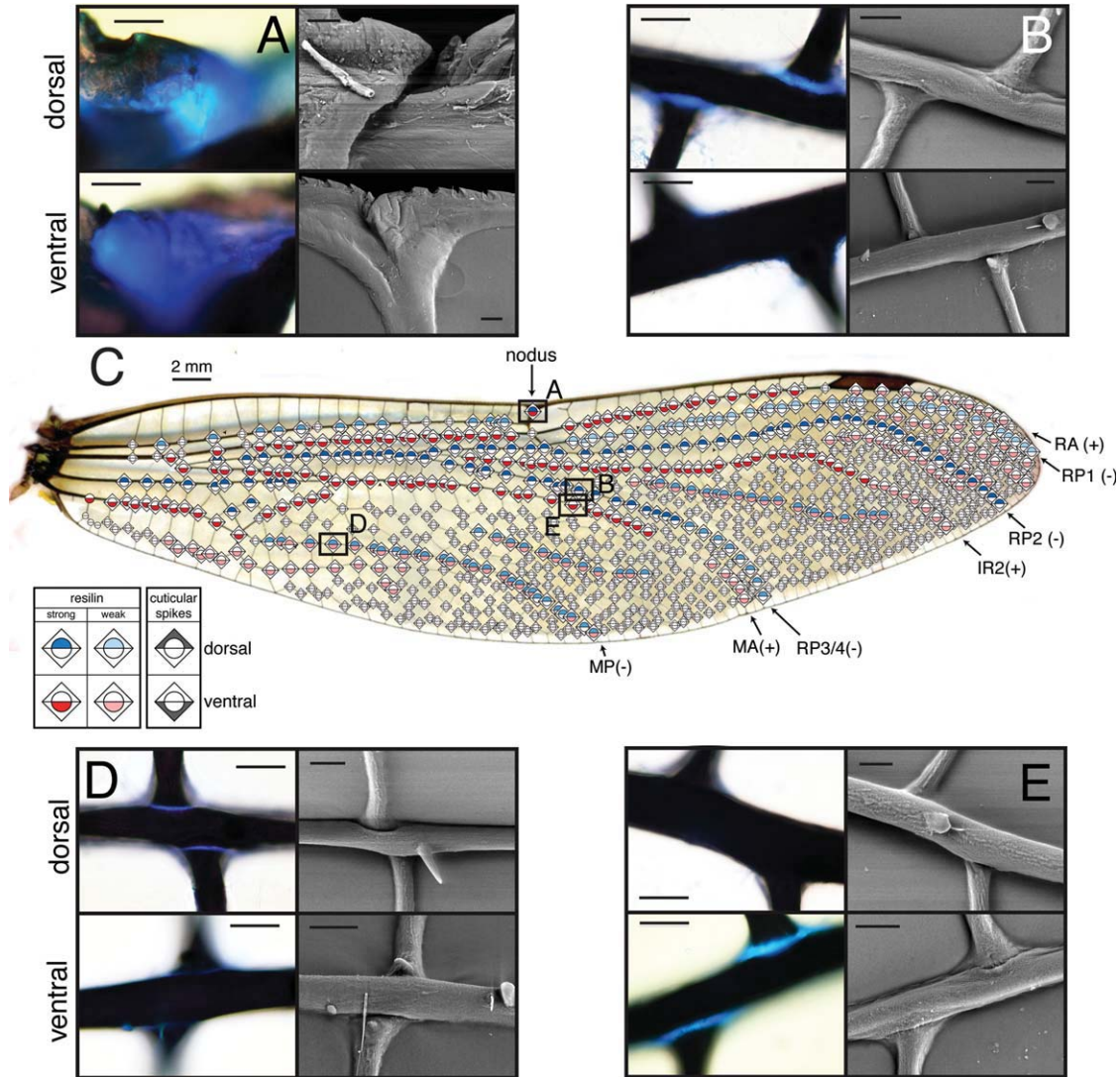


Fig. 7. Dorsal and ventral distribution of resilin pads and cuticular spikes in the vein-joints of a forewing from the dragonfly *A. verticalis*. For further explanation of symbols, see Figure 4 legend. **A,B**: FM (DAPI filter + brightfield light) and SEM images of both the dorsal and ventral sides of selected joints. Scale bars represent 25 μ m. **A**: The nodus has a large pad of resilin on both the dorsal and ventral sides. **B**: Dorsal resilin only. **C**: Photograph of a wing on which the condition of the each joint has been recorded. Scale bar represents 2 mm. **D**: Weak dorsal and ventral resilin. **E**: Ventral resilin only.

By contrast, among Zygoptera (damselflies), longitudinal veins are characterized more by double-sided vein-joints that contain resilin both dorsally and ventrally (Fig. 9A, Groups 2–4). Beyond this general similarity, however, resilin patterns between different taxa within the damselflies are more variable than within dragonflies. *Ischnura* and *Enallagma* appear to have a dragonfly-like pattern, but with additional ventral resilin on veins RP2 and RP3/4 (Fig. 9A, Group 2). The *Calopteryx* pattern has double-sided resilin joints on all the veins in the posterior of the trailing edge (Fig. 9A, Group 3), whereas *Lestes* had a distinct pattern of veins alternating between no resilin and those with both dorsal and ventral resilin (Fig. 9A, Group 4).

Spikes. Similar to resilin, joint-associated spikes are widespread within the Odonata; they were present in all but two of the examined species (Fig. 9A). Notably, the specific morphology of spikes differed between the two suborders; when present, spikes tended to point toward the adjacent longitudinal vein more often in damselflies (as in Fig. 8B), whereas in dragonflies they tended to point directly away from the wing surface (as in Fig. 8A). Yet, in general, overall patterns of spike placement were much more variable between groups than resilin patterns.

On the whole, joint-associated spikes were not found to be a major factor in dragonfly vein-joints. Spikes were completely absent in *Aeshna* (although there are some protrusions that, while

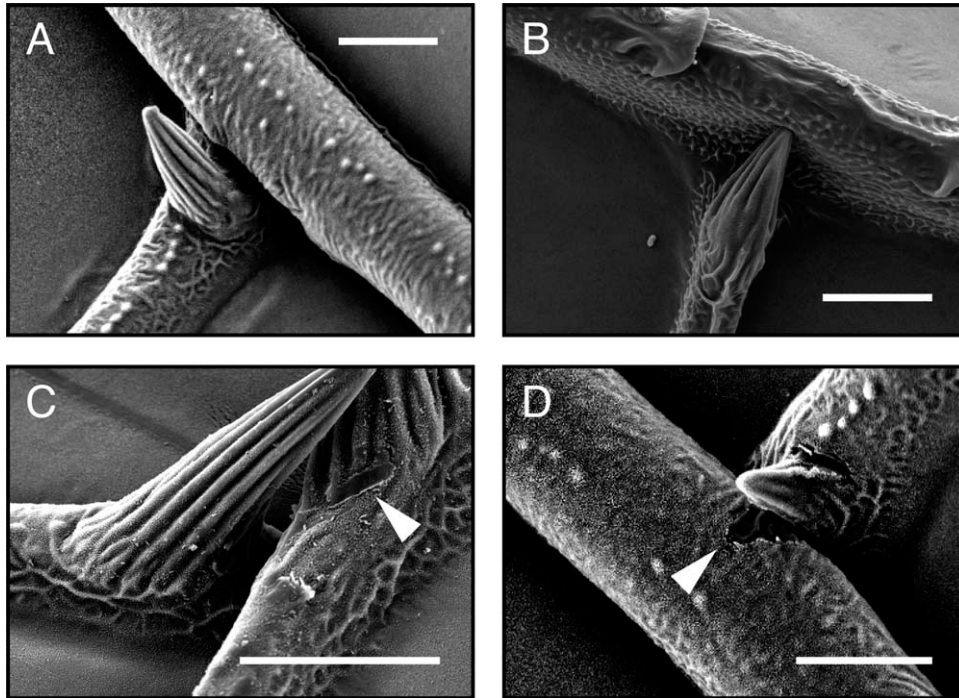


Fig. 8. Relation of cuticular spikes with veins. Sample SEM images illustrating (A) a spike pointing directly away from the wing surface and (B) a spike orientated toward the adjacent longitudinal vein. C,D: Spikes that are adjacent to a longitudinal vein where an imprint in the waxy coating on the vein is visible (see arrowheads). Scale bars represent 20 μm .

too small to interact significantly with the wing, otherwise resemble what we term spikes; see Fig. 7D for an example) and are sometimes present in the trailing edge region among the rest of the dragonfly genera we examined (Fig. 9A, Group 1). Spikes appear to play a larger role in the structure of damselfly wings. *Ischnura* and *Enallagma* share a crisp pattern: spikes are found only on the ventral side of RP1, RP2, RP3/4, and MP (Fig. 9A, Group 2). *Calopteryx* has spikes on both the dorsal and ventral side of RP2, RP3/4, MA, and MP (Fig. 9A, Group 3). In *Lestes*, the spikes were present, but there was no clearly discernable pattern to their distribution (Fig. 9A, Group 4).

Mechanical Tests

In vein-joint pivoting experiments on isolated joints from the damselfly *I. verticalis*, double-sided resilin joints (from vein RP2) have significantly lower rotational stiffness than single-sided resilin joints (from vein IR2, immediately adjacent to RP2). Two individual joints were prepared from each vein, and in each case the rotational stiffness was measured repeatedly across a range of forces applied to the cross-vein that connects with the vein-joint. The cross-vein was always pivoted in the direction that would disengage joint-associated spikes. We found that vein-joints on RP2 had a mean rotational stiffness of 9.7×10^{-9} N/degree

(SD = 1.5×10^{-9} N/degree), whereas those from IR2 had a mean rotational stiffness of 5.8×10^{-8} N/degree (SD = 1.2×10^{-8} N/degree). Thus, the single-sided resilin joints were approximately six times stiffer than neighboring double-sided joints (Student's *t*-test; $P \ll 0.05$).

Wing movement around individual longitudinal veins within intact wings also showed different degrees of deformation in response to whole-wing loading according to their resilin and joint-associated spike morphology. When stressed from the ventral side of the wing (thus, disengaging the joint-associated spikes and isolating the mechanical function of resilin patches), wing material pivoted significantly farther about both double-sided resilin veins (RP2 and RP3/4) than about the vein containing minimal resilin (IR2; Student's *t*-test, $P < 0.05$; Fig. 4C). Between the two double-sided resilin veins, there was significantly more pivoting about RP2 than RP3/4 ($P < 0.05$; Fig. 4C).

DISCUSSION

We have applied a systematic, comparative approach to characterize two wing morphological features that had been described previously in a limited number of species (Newman, 1982; Gorb, 1999). This approach revealed that these two features, resilin patches, and cuticular spikes, are extremely common within the Odonata, yet the

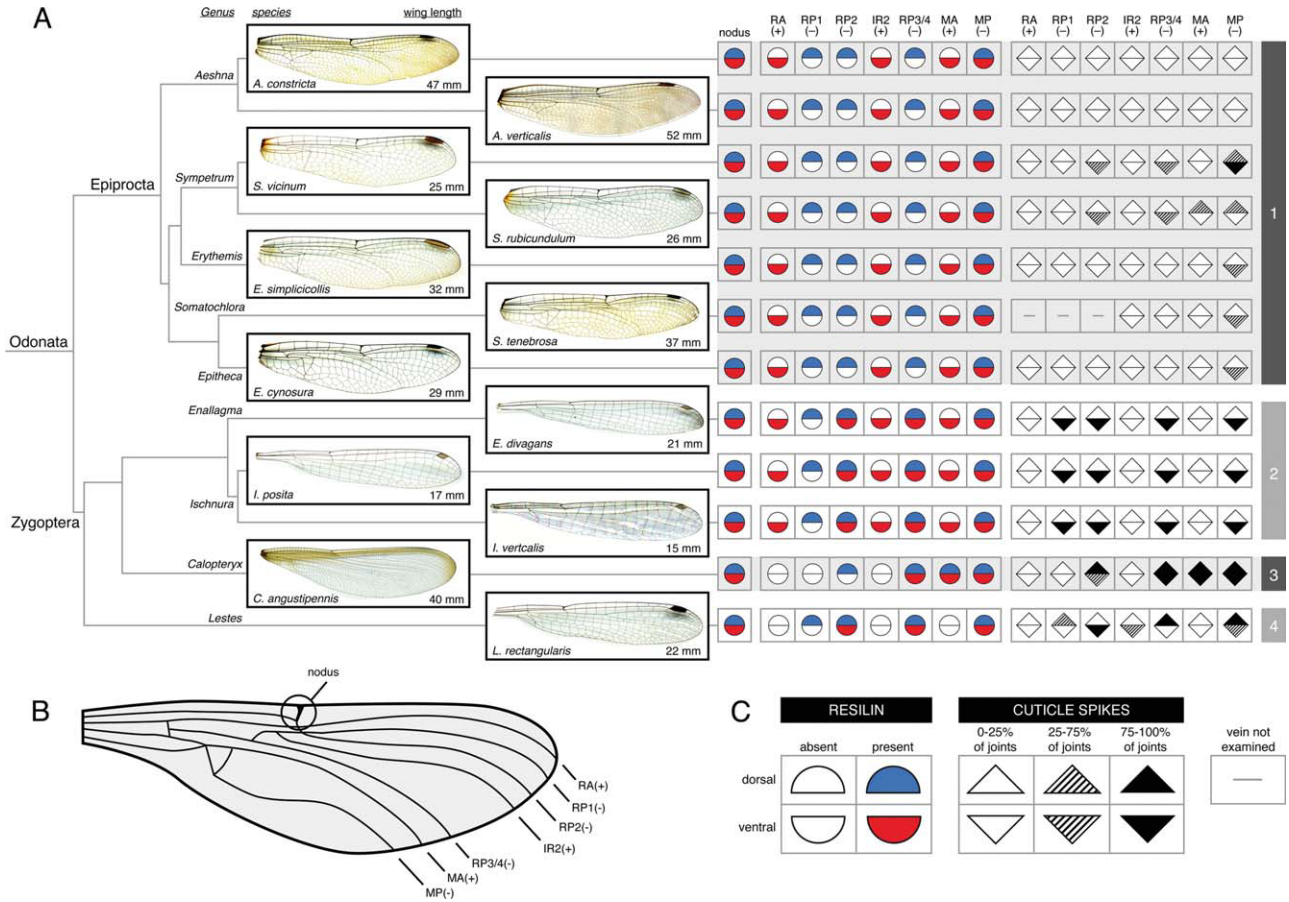


Fig. 9. **A**: Phylogenetic comparison of resilin and cuticular spike patterns in Odonata. Branches show the relationship of taxa based on the combined molecular and morphological phylogeny by Bybee et al. (2008). Major differences in overall morphological patterns are highlighted by shaded groups labeled 1–4. (1) Suborder: Epiprocta (dragonflies); (2–4) Suborder: Zygoptera (damselflies). **B**: Veins were identified following the system of Riek and Kukalova-Peck (1984). **C**: Key to the symbols used in Panel A. Veins were marked as “present” for resilin if any of their vein-joints showed visible blue fluorescence, and marked as “absent” if not (see text for scoring details).

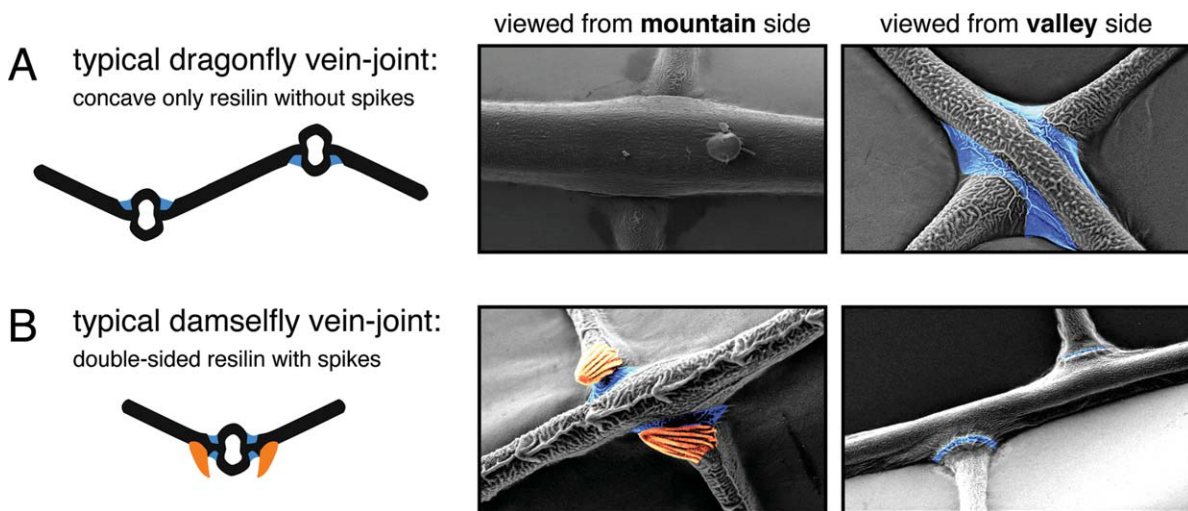


Fig. 10. Schematic illustrations of major vein-joint types with representative false color SEMs. Joints of Type A are more common in dragonflies (Epiprocta), whereas joints of Type B are more common in damselflies (Zygoptera).

varied arrangements of the two structures within wings follow particular phylogenetic patterns. We observed two especially common patterns of resilin morphology. In one of these, "concave only," resilin was found on the ventral side of mountain veins (indicated by a "+" in Figs. 5–7 and 9) or on the dorsal side of valley veins (indicated by a "-" in Figs. 5–7 and 9). A cross-sectional schematic is shown in Figure 10A. In the other pattern, "double-sided with spikes," resilin was visible on both sides of a vein-joint and cuticle spikes were present on only the dorsal or ventral side. This is schematized in Figure 10B. The former pattern is characteristic of dragonflies (Ephemeroptera), whereas the latter is more characteristic of damselflies (Zygoptera).

These morphological differences between dragonflies and damselflies may reflect the distinct kinematics of these two groups. Dragonflies typically use an inclined stroke plane (Newman, 1982; Ruppell, 1989; Wakeling and Ellington, 1997), whereas the stroke plane of damselflies is usually closer to horizontal (Newman, 1982; Ruppell, 1989; Sato and Azuma, 1997), characteristic of "normal hovering" (Weis-Fogh, 1973). Double-sided resilin joints in damselflies may therefore reflect the relative symmetry between upstroke and downstroke in this group. This hypothesis remains conjectural, however, as we have no direct evidence that double-sided resilin joints contribute equally to dorsal and ventral flexibility. Damselflies also significantly exhibit higher levels of wing twisting during stroke reversal than dragonflies and this difference is correlated with the position of the nodus in Odonata (Wootton and Newman, 2008). The high degree of conservation of resilin enrichment in the nodus (Fig. 9A) supports the hypothesis that it plays a key role in enabling wing torsion.

The Mechanical Function of Resilin Joints in Odonata

Our mechanical evidence supports the standing hypotheses that 1) some vein-joints are more flexible than others (Newman, 1982) and 2) the pattern of resilin distribution is one of the several mechanisms that determine the mechanical attributes of the wing (Gorb, 1999). Although single-joint and whole-wing bending tests were performed on different species, the high degree of conservation of both resilin and spike patterns between the *Ischnura* and *Enallagma* genera (Fig. 9) is strong evidence that pattern and mechanical function of joints is similar between the two examined species.

Flexible vein-joints appear to provide chordwise wing flexibility while maintaining spanwise stiffness. Combes and Daniel (2003) demonstrated that flexural stiffness across the span of insect wings is regularly one to two orders of magnitude larger than flexural stiffness across the chord. Flexible vein-joints probably play a critical role in estab-

lishing this difference between spanwise and chordwise flexural stiffness in odonate wings, by either of two possible mechanisms, each of which is consistent with the findings of Gorb (1999) for the damselfly *E. cyathigerum*. In the first mechanism (shown in Fig. 11A), longitudinal veins that contain resilin vein-joints alternate with those that do not. Longitudinal veins containing flexible joints would function as flexion lines for chordwise flexibility, while the wing is supported across the span by the stiffer longitudinal veins.

In the second mechanism, chordwise flexibility is produced by flexible patches flanking longitudinal veins (see Fig. 11B). As the relatively rigid longitudinal veins are structurally intact and not interrupted by resilin patches, they will resist flexion along the span of the wing, while mobility in the joints where cross-veins meet the longitudinal veins allows the wing to flex along the chord. Whereas the first mechanism is a product of the spatial arrangement of flexible joints within the matrix of vein-joints, the second mechanism is a product of the specific, local morphology at vein-joints.

Patterns of flexibility in dragonflies are probably a product only of the latter mechanism, because there is no alternation between flexible and stiff longitudinal veins (Fig. 9A, Group 1). Damselflies, however, may use both mechanisms. There is some alternation between flexible and stiff longitudinal veins (Fig. 5C; Gorb, 1999). However, as Gorb (1999) first noted, the morphology at flexible vein-joints resembles the second mechanism (Fig. 11B), with patches of resilin running parallel to but rarely crossing longitudinal veins (see Fig. 7 for some examples).

Previous work has shown that spanwise and chordwise flexural stiffness of the wings of a wide array of insects follows a strong positive allometric scaling pattern with wing size across both the span and chord (Combes and Daniel, 2003). As a consequence of differential scaling across the span and chord (Combes and Daniel, 2003), the difference between spanwise and chordwise flexural stiffness will tend to be greater for larger insects. The demands for structural anisotropy are therefore especially strong for the wings of the largest insects, and may have been an important factor in the morphological evolution of gigantic, extinct Odonoptera (e.g., Protodonata, with wing spans around 60 cm).

The Mechanical Function of Cuticular Spikes in Odonata

Newman (1982) first observed joint-associated spikes in odonate wings and proposed that these joints function as "joint-stoppers" that inhibit the movement of cross-veins around a longitudinal vein after a certain amount of rotation. This expla-

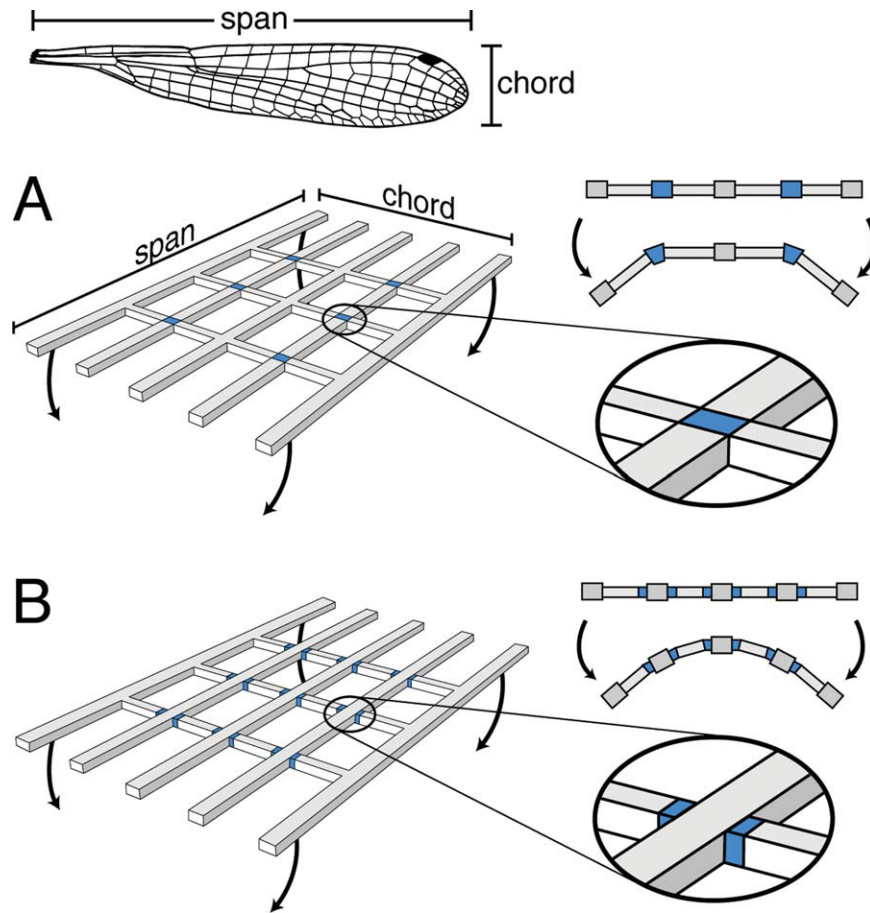


Fig. 11. Two models for how flexible vein-joints could enable the odonate wing to be flexible across the chord of the wing, but relatively stiff along the span of the wing. **A:** Flexible vein-joints confer general mobility to a vein, but they are present only in alternating longitudinal veins, allowing the intervening veins to provide spanwise stiffness. **B:** Flexible material flanks the longitudinal vein at each flexible vein-joint, thereby restricting mobility to chordwise movement.

nation seems plausible for several of the genera we examined (particularly *Enallagma* and *Ischnura*), but less probably for some spike morphologies, especially those in which the spikes grow directly into the adjacent longitudinal vein. Rather, it may be that these “fixed” spikes (such as in *Calopteryx*, where joint-associated spikes are exclusively found along resilin-containing veins) render an otherwise flexible joint rigid. In this sense, cuticular spikes could provide an additional mechanism for adjusting the flexural stiffness of the insect wing.

Although they may be serving these functions in some groups, it is clear that joint-associated spikes are not essential for all odonates. An interesting case is the genus *Aeshna* within the Eiprocta (see Fig. 7). Although there are prominent spikelike cuticular structures present along many of the wing veins, none of them are found associated with vein-joints. Although cuticular spikes are widespread on the wings of Odonata (see D’Andrea and Carfi, 1988, 1989), it is unclear whether the absence of joint-associated spikes in *Aeshna* represents an ancestral condition or a subsequent loss.

The Evolutionary Context of Wing Flexibility

If changes in insect wing size and shape over evolutionary time engender necessary shifts in flexural stiffness, this raises the question: what specific morphological changes might underlie general shifts in wing flexural stiffness? The answer is not entirely clear. Namely, it is unknown whether changes in wing flexibility occur throughout the wing (i.e., changing evenly throughout the wing as a product of changes in overall thickness or material properties) or at discrete locations in the wing. Our results support the idea that in odonates, wing flexibility can be regulated, at least in part, by morphologies at discrete locations in the wing (i.e., specific longitudinal veins).

The idea that natural selection would act on discrete units within the wing agrees well with what is understood about evolutionary changes in wing morphology throughout the insects. For example, in wild *Drosophila guttifera*, co-option of a pre-existing *cis*-regulatory region has created a gene expression pattern that is completely limited to the locations on the wing where sensory organs

and cross-veins meet longitudinal veins (Werner et al., 2010). One can imagine that a similar process might have been present within Odonata, responsible for the advent of resilin and cuticle spike placement specifically at vein-joints.

ACKNOWLEDGMENTS

The authors thank B. Clark, S. DiNardo, S. Mukherjee, D. Rundle, J. Iwasaki, B. Roberts, and Swarthmore College's 2010 Invertebrate Biology class for comments on earlier drafts of this work. They also thank S. Gorb for useful advice during the beginning stages of this project, P. Christmas for early contributions to this work, and D. Smith, E. Lavoie-Hodges, and A. Graham for sample preparation and help with microscopy.

LITERATURE CITED

- Anderson SO. 1963. Characterization of a new type of cross-linkage in resilin, a rubber-like protein. *Biochim Biophys Acta* 69:249–262.
- Bennet-Clark HC, Lucey ECA. 1967. The jump of the flea: A study of the energetics and a model of the mechanism. *J Exp Biol* 47:59–76.
- Biewener AA, Dial KP. 1995. In vivo strain in the humerus of pigeons (*Columba livia*) during flight. *J Morph* 225:61–75.
- Burrows M, Shaw SR, Sutton GP. 2008. Resilin and chitinous cuticle form a composite structure for energy storage in jumping by froghopper insects. *BMC Biol* 6:41–56.
- Bybee SM, Ogden TH, Branham MA, Whiting MF. 2008. Molecules, morphology and fossils: A comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics* 24:477–514.
- Cartar RV. 1992. Morphological senescence and longevity: An experiment relating wing wear and life span in foraging wild bumble bees. *J Anim Ecol* 61:225–231.
- Combes SA, Crall JD, Mukherjee S. 2010. Dynamics of animal movement in an ecological context: Dragonfly wing damage reduces flight performance and predation success. *Biol Lett* 6:426–429.
- Combes SA, Daniel TL. 2003. Flexural stiffness in insect wings. I. Scaling and influence of wing venation. *J Exp Biol* 206:2979–2987.
- D'Andrea M, Carfi S. 1988. Spines on the wing veins in Odonata. 1. Zygoptera. *Odonatologica* 17:313–335.
- D'Andrea M, Carfi S. 1989. Spines on the wing veins in Odonata. 2. Anisozygoptera and anisoptera. *Odonatologica* 18:147–178.
- Daniel TL, Combes SA. 2002. Flexing wings and fins: Bending by inertial or fluid-dynamic forces? *Int Comp Biol* 42:1044–1049.
- Emler RB. 1982. Echinoderm calcite: A mechanical analysis from larval spicules. *Biol Bull* 163:264–275.
- Ennos RA. 1988. The importance of torsion in the design of insect wings. *J Exp Biol* 140:137–160.
- Faupel JH. 1964. *Engineering Design, A Synthesis of Stress Analysis and Materials Engineering*. New York: Wiley and Sons.
- Fraenkel G, Hsiao C. 1965. Bursicon, a hormone which mediates tanning of the cuticle in the adult fly and other insects. *J Ins Physiol* 11:513–556.
- Gorb SN. 1999. Serial elastic elements in the damselfly wing: Mobile vein joints contain resilin. *Naturwissenschaften* 86:552–555.
- Haas F, Gorb S, Blickhan R. 2000a. The function of resilin in beetle wings. *Proc Roy Soc Lond* 267:1375–1381.
- Haas F, Gorb S, Wootton RJ. 2000b. Elastic joints in dermapteran hind wings: Materials and wing folding. *Arthropod Struct Dev* 29:137–146.
- Hedenström A, Ellington CP, Wolf TJ. 2001. Wing wear, aerodynamics and flight energetics in bumblebees (*Bombus terrestris*): An experimental study. *Funct Ecol* 15:417–422.
- Jensen M, Weis-Fogh T. 1962. Biology and physics of locust flight. V. Strength and elasticity of locust cuticle. *Phil Trans Roy Soc B* 245:137–169.
- Kingsolver JG. 1999. Experimental analyses of wing size, flight, and survival in the western white butterfly. *Evolution* 53:1479–1490.
- Mountcastle AM, Daniel TL. 2009. Aerodynamic and functional consequences of wing compliance. *Exp Fluids* 46:873–882.
- Neff D, Frazier SF, Quimby L, Wang RT, Zill S. 2000. Identification of resilin in the leg of cockroach, *Periplaneta americana*: Confirmation by a simple method using pH dependence of UV fluorescence. *Arthropod Struct Dev* 29:75–83.
- Newman DJS. 1982. *The Functional Wing Morphology of Some Odonata* [dissertation]. Exeter: University of Exeter. 281 pp.
- Newman DJS, Wootton RJ. 1986. An approach to the mechanics of pleating in dragonfly wings. *J Exp Biol* 125:361–372.
- Rehn AC. 2003. Phylogenetic analysis of higher-level relationships of Odonata. *Syst Entomol* 28:181–239.
- Riek EF, Kukalova-Peck J. 1984. A new interpretation of dragonfly wing venation based upon Early Upper Carboniferous fossils from Argentina (Insecta: Odonatoidea) and basic character states in pterygote wings. *Can J Zool* 62:1150–1166.
- Rüppell G. 1989. Kinematic analysis of symmetrical flight manoeuvres of Odonata. *J Exp Biol* 144:13–42.
- Sato M, Azuma A. 1997. The flight performance of a damselfly *Ceriatron melanurum* Selys. *J Exp Biol* 200:1765–1779.
- Swartz SM, Bennett MB, Carrier DR. 1992. Wing bone stresses in free flying bats and the evolution of skeletal design for flight. *Nature* 359:726–729.
- Vincent JFV, Wegst UGK. 2004. Design and mechanical properties of insect cuticle. *Arthropod Struct Dev* 33:187–199.
- Wakeling J, Ellington C. 1997. Dragonfly flight. II. Velocities, acceleration, and kinematics of flapping flight. *J Exp Biol* 200:557–582.
- Weis-Fogh T. 1960. A rubber-like protein in insect cuticle. *J Exp Biol* 37:889–907.
- Weis-Fogh T. 1973. Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. *J Exp Biol* 59:169–230.
- Werner T, Koshikawa S, Williams TM, Carroll SB. 2010. Generation of a novel wing colour pattern by the Wingless morphogen. *Nature* 464:1143–1148.
- Wootton RJ. 1981. Support and deformability in insect wings. *J Zool Lond* 193:447–468.
- Wootton RJ. 1991. The functional morphology of the wings of Odonata. *Adv Odonatol* 5:153–169.
- Wootton RJ. 1992. Functional morphology of insect wings. *Annu Rev Entomol* 37:113–140.
- Wootton RJ, Kukalova-Peck J, Newman DJS, Muzon J. 1998. Smart engineering in the mid-carboniferous: How well could palaeozoic dragonflies fly? *Science* 282:749–751.
- Wootton RJ, Newman DJS. 2008. Evolution, diversification, and mechanics of dragonfly wings. In: Cordoboa-Aguilar A, editor. *Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research*. Oxford University Press: New York. pp. 261–274.
- Young J, Walker SM, Bompfrey RJ, Taylor GK, Thomas ALR. 2009. Details of insect wing design and deformation enhance aerodynamic function and flight efficiency. *Science* 325:1549–1552.
- Zhao L, Huang Q, Deng X, Sane SP. 2010. Aerodynamic effects of flexibility in flapping wings. *J Roy Soc Int* 7:485–497.