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

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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 Ephemeroptera) 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. 000:000–000, 2011. © 2011 Wiley-Liss, 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.
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 junctions 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 froghopper 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 Weis-Fogh, 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.
one specimen each of nine additional species, drawing from this time focusing solely on longitudinal veins. We examined conducted a wider taxonomic comparison of resilin patterns, wing homology system (as updated by Rehn, 2003). Next, we Veins were identified using the Riek and Kukalova-Peck (1984) Fluorescence Microscopy the wings of the damselfly DMRB fluorescent microscope up to a magnification of 40×/C24 nm, thereby narrowly selecting resilin’s known emission peak of UV excitation at 330–370 nm and allows emission at 410–450 nm for the presence of resilin in a subset of wings by taking mono- a Zeiss Axioscope digital camera. We provided further evidence on both the dorsal and ventral sides. Images were captured with deep blue color under UV excitation (see, for instance, Fig. 2A), tion 340–380 nm, 420 nm longpass emission filter). Wing veins DMRB or a Zeiss Axioplan microscope with a DAPI filter (excitation for the presence of resilin in a subset of wings by tissue imaging (see Fig. 2B). Using this method, we were able to image veins and vein-joints with high specificity and sensitivity, allowing for a detailed understanding of resilin distribution in dragonfly and damselfly wings.

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 monochromatic 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×. 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, Sympteurum rubicundulum, Erythemis simplicicollis, Somatochlora tenebrosa, Epitheca 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 matched 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).
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:

\[ F = \frac{3 \times EI \times \delta}{L^3} \]  

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, δL < 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:

\[ \text{rotational stiffness (N m/degree)} = F \times d/\theta \]  

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 on a diet of brine shrimp. After emer-

![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.](image-url)
the insect pin and after the wing had rotated through an angle.

The majority of deformation was occurring across the chord, but flexible across the chord than across the span, we assumed that 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 of 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. 6D and 7D) or neither side of the joint (e.g., Fig. 5E).

Scanning electron micrographs show that odo-nate 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...
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,
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).
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
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 \(9.7 \times 10^{-9} \text{ N/degree}\) (SD = \(1.5 \times 10^{-9} \text{ N/degree}\)), whereas those from IR2 had a mean rotational stiffness of \(5.8 \times 10^{-8} \text{ N/degree}\) (SD = \(1.2 \times 10^{-8} \text{ N/degree}\)). Thus, the single-sided resilin joints were approximately six times stiffer than neighboring double-sided joints (Student’s t-test; \(P << 0.05\)).

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 (Epiprocta), 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; Rüppell, 1989; Wakeling and Ellington, 1997), whereas the stroke plane of damselflies is usually closer to horizontal (Newman, 1982; Rüppell, 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 establishing 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 Odonatoptera (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-
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 Epiprocta (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

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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.
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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.

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LITERATURE CITED


Swartz SM, Bennett MM, Carrier DR. 1992. Wing bone stresses responsible for the advent of resilin and cuticle form a composite structure for energy storage in jump-