The function of resilin in beetle wings

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This account shows the distribution of elastic elements in hind wings in the scarabaeid *Pachnoda marginata* and coccinellid *Coccinella septempunctata* (both Coleoptera). Occurrence of resilin, a rubber-like protein, in some mobile joints together with data on wing unfolding and flight kinematics suggest that resilin in the beetle wing has multiple functions. First, the distribution pattern of resilin in the wing correlates with the particular folding pattern of the wing. Second, our data show that resilin occurs at the places where extra elasticity is needed, for example in wing folds, to prevent material damage during repeated folding and unfolding. Third, resilin provides the wing with elasticity in order to be deformable by aerodynamic forces. This may result in elastic energy storage in the wing.

Keywords: wing; Insecta; flight; vein joint design; material composition; resilin

1. INTRODUCTION

The evolution of flight was a key event in insect evolution. This innovation among others let insects diversify on an unprecedented scale. Wings are complex structures, which are designed to enable controlled torsion, essential to flapping flight. Although wing morphology and flight types diversified enormously, the general principle of insect flight remained the same. Flight muscles are located in the thorax proper, whereas the wing proper is completely without muscles.

These circumstances have two implications. First, folding of any wing part cannot be achieved by intrinsic muscles, but must be done by either extrinsic, thoracic muscles or intrinsic elasticity. Many insects, such as earwigs and beetles, have developed complex mechanisms of wing folding (Haas 1995; Haas & Wootton 1996), which are advantageous for insects living in soil or other narrow crevices. The folding pattern depends on the venation pattern and the material properties of the structures involved. Consequently, the design of wings in Coleoptera has an additional function compared with normal wings. Their design is a compromise between flight and folding (Brackenbury 1994).

Second, the striking deformations of wings in flight result from thoracic muscle forces combined with inertial and aerodynamic forces. This is in contrast to birds' flight in which the wing shape is partly controlled by intrinsic muscles. The wings are much less deformed by aerodynamic forces. Previous studies on the functional morphology of insect wings have shown that the camber and angle of attack are automatically maintained under aerodynamic loading by a set of internal mechanisms including vein pattern, vein curvature and joints between cross and longitudinal veins (Ennos 1988; Wootton 1991).

Our previous results on the coleopteran wing design (Haas 1998) suggest that the type and distribution of materials are essential to both folding and aerodynamics.

In general, wings consist of a skeletal network of relatively rigid veins, which are interconnected by thin membranous areas. This network is three-dimensional through the veins changing from convex to concave in the wing. Both veins and membrane consist of a double layer of cuticle, a biological fibrous composite material (Neville 1993), with mechanical properties ranging from very stiff to flexible depending on its chemical composition (Hackman & Goldberg 1987; Hepburn & Chandler 1976).

Veins of an insect wing may be movably connected with other veins, or firmly fused (Newman 1982). The vein articulations are small joints with particular turning axes and working zones. Local displacements in mobile vein joints are responsible for the variable wing profile during flight.

Our study reveals the presence of the rubber-like protein resilin within some flexible joints of hind-wing veins and in areas of the wing membrane in the scarabaeid *Pachnoda marginata* and coccinellid *Coccinella septempunctata* (both Coleoptera). The distribution pattern of resilin in the middle and distal regions of the beetle wings together with the data on wing folding and flight kinematics suggest an important role for these elastic springs in the wing deformation during wing folding and flight.

2. MATERIAL AND METHODS

(a) *Species*

Representatives of two coleopteran families were used. The families differ in wing design and wing-folding pattern. The scarabaeid *P. marginata* Kolbe, 1906 (27 mm hind-wing length) were obtained from laboratory culture. The coccinellid *C. septempunctata* (Linnaeus, 1758) (10 mm hind-wing length) were captured in the field in central Germany.

(b) *Flight kinematics*

Video sequences were taken with a Sony DCR VX 1000 E digital video camera (Sony, Tokyo, Japan) with a Nikon 3T close-up lens (Nikon, Tokyo, Japan). The outdoor sequences were taken with the shutter speed manually set to 0.1 ms, whereas in the laboratory sequences a Metz 50 MZ 5 flash (Metz, Fürth, Germany) with stroboscope set to 50 Hz was used. Shutter
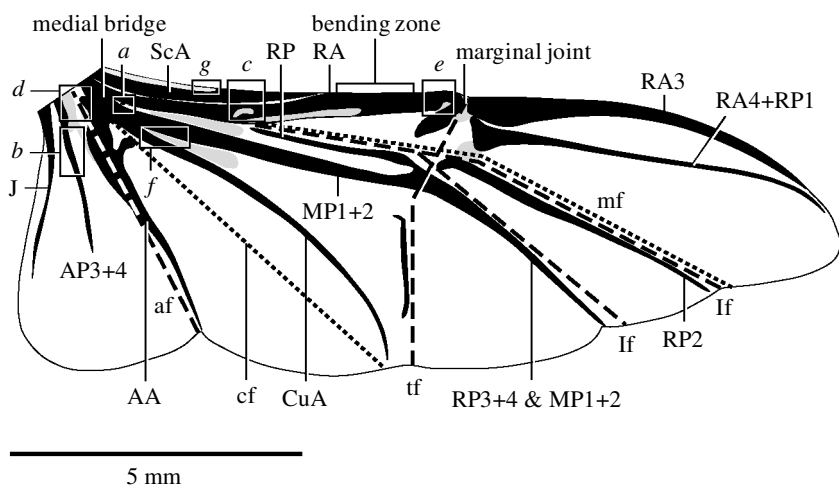
Figure 1. *Pachnoda marginata*, left hind wing showing veins, resilin-containing areas and major folding and flexion lines. Digitized from a microscope slide. Terminology after Kukalová-Peck & Lawrence (1993). Rectangles *a–g* indicate joints shown in figure 2. AA, anal anterior; CuA, cubitus anterior; af, anal fold; cf, claval fold; J, jugal; lf, longitudinal fold; mf, median flexion line; MP1 + 2, media posterior; AP3 + 4, anal posterior; RA, radius anterior; RP, radius posterior; ScA, subcosta anterior; tf, transverse fold.
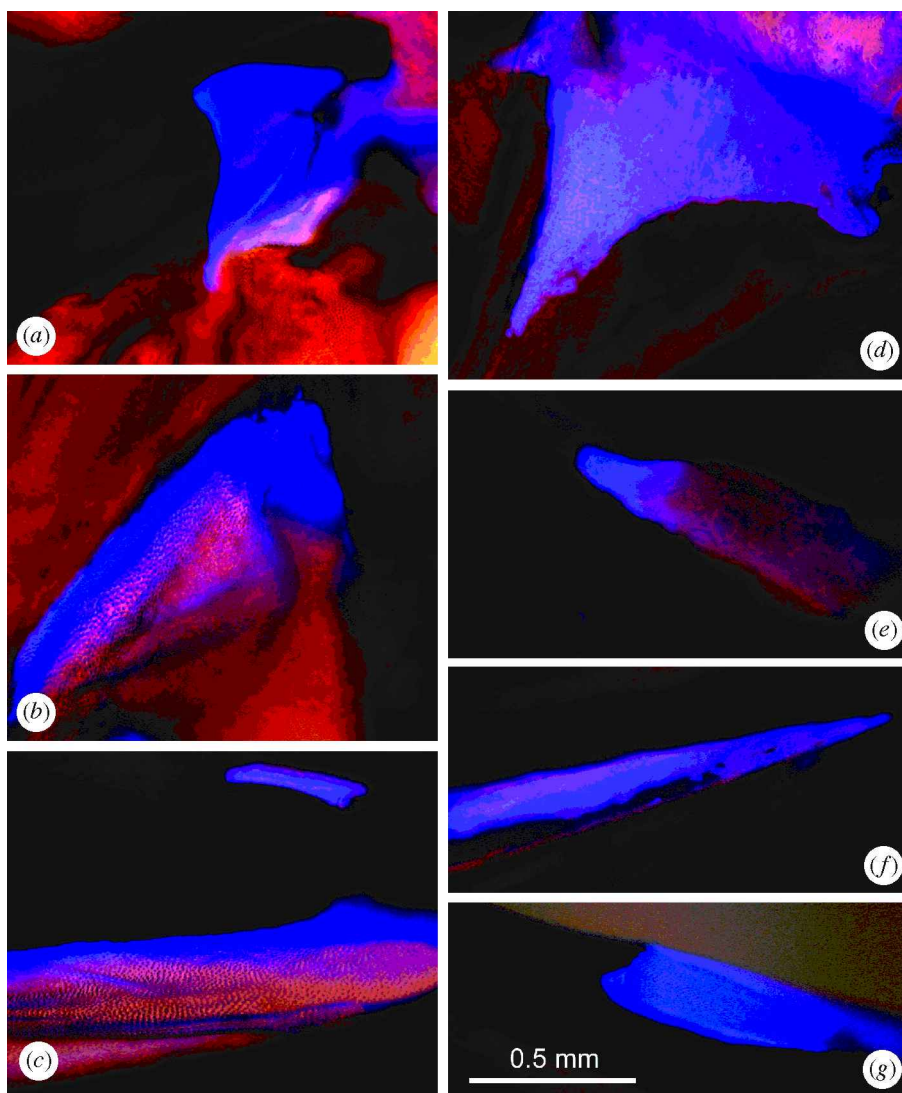

Figure 2. *Pachnoda marginata*, vein joints with the rubber-like protein resilin (fluorescent microscopy combined with bright-field light microscopy). Blue to violet areas contain resilin. For location of the regions in the wing see figure 1. (*a*) Very basal region of the wing, (*b*) basal region between the anal anterior (AA) and AP3 + 4, (*c*) region between the radius anterior (RA) and radius posterior (RP), (*d*) very basal region of the wing, (*e*) region of the marginal joint of the wing, (*f*) region between the subcosta anterior (ScA) and media posterior (MP1 + 2), (*g*) basal region of the costal wing margin.
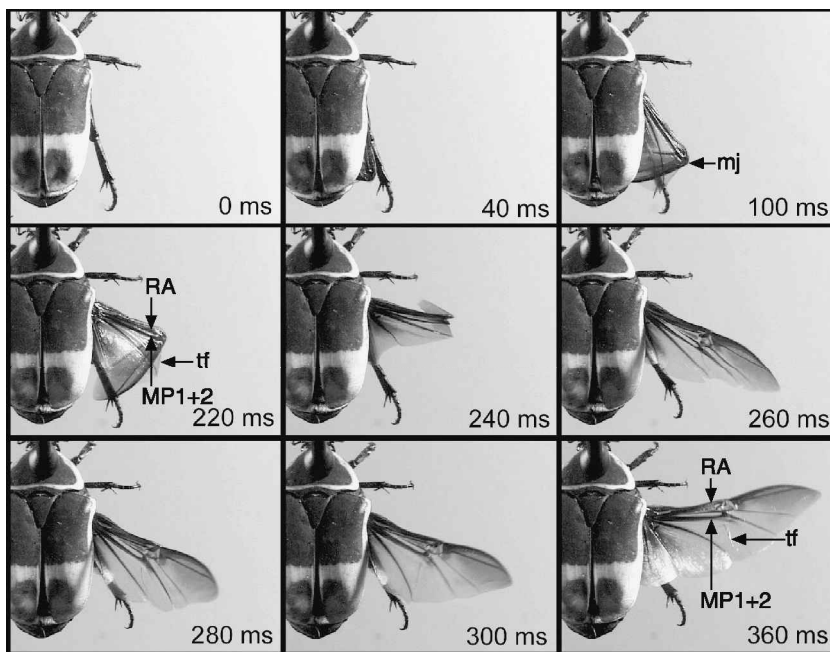
Figure 3. *Pachnoda marginata*, video sequence of the wing unfolding, tethered beetle, dorsal aspect. mj, marginal joint; tf, transverse fold; RA, radius anterior; MP1 + 2, media posterior.
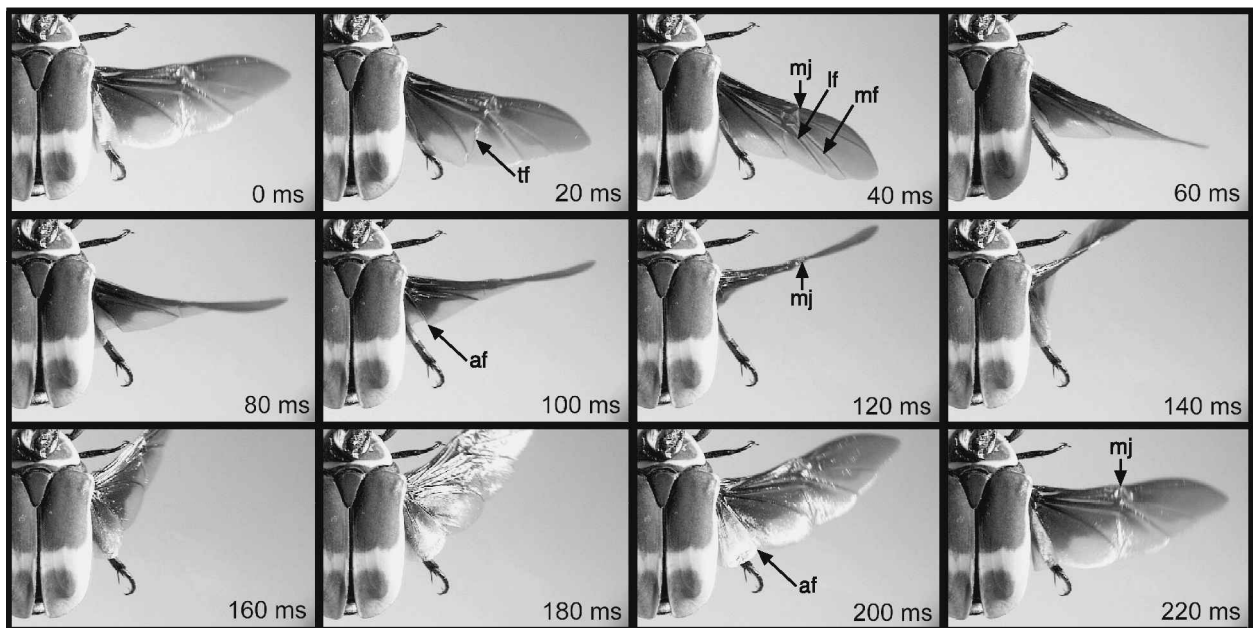

Figure 4. *Pachnoda marginata*, video sequence of single cycle of the wing beat, tethered beetle, dorsal aspect. af, anal fold; lf, longitudinal fold; mf, median flexion line; mj, marginal joint; tf, transverse fold.

speed was manually set to 20 ms and the flash time was 0.1 ms. The half frames were separated in the computer to increase time resolution to 20 ms. Some frames have been omitted in the plates, the time-scale is given there.

Additional photographs were taken with 35 mm equipment (Nikon FE-2, 105 mm Micro-Nikkor lens, Nikon 3T close-up lens) at life size and flash at 0.1 ms (two synchronized Metz 45 CT 4 flash lights).

(c) *Bright-field light microscopy and fluorescent microscopy*

Wings of freshly killed specimens or those fixed in 70% ethanol and rehydrated for 24–48 h were used. The wings were cut off, mounted on cover-slips in a water-soluble medium (Moviol; Hoechst, Frankfurt, Germany), and observed under a fluorescent

microscope (Zeiss Axioscop; Zeiss, Oberkochen, Germany) in bright field or in one of three bands of wavelengths: the green (excitation 512–546 nm, emission 600–640 nm), red (excitation 710–775 nm, emission 810–890 nm) and ultraviolet (UV) band (excitation 340–380 nm, emission 425 nm). It is known that insect cuticle has a strong autofluorescence at wavelengths from blue-green to deep-red. However, resilin has an autofluorescence at a very narrow band of wavelengths around 400 nm (Andersen & Weis-Fogh 1964). Resilin appears blue in our figures. This property makes it possible to reveal resilin in biologically native structures without immune labelling or other treatments.

(d) *Image processing*

Two different procedures for picture acquisition were used. First, pictures were taken using fluorescence microscopy (UV
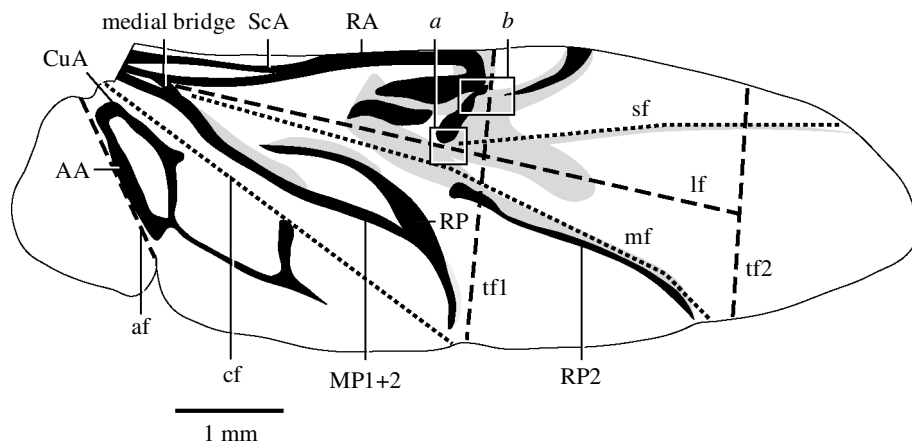
Figure 5. *Coccinella septempunctata*, left hind wing showing veins, resilin-containing areas and major folding and flexion lines. Digitized from a microscope slide. Terminology after Kukalová-Peck & Lawrence (1993). Rectangles *a* and *b* indicate joints shown in figure 6. AA, anal anterior; CuA, cubitus anterior; af, anal fold; cf, claval fold; lf, longitudinal fold; mf, median flexion line; sf, stiffening fold, visible only in flight and in fluorescent microscope; MP1 + 2, media posterior; RA, radius anterior; RP, radius posterior; ScA, subcosta anterior; tf1, first transverse fold; tf2, second transverse fold.

band) and bright-field microscopy of the same wing area and afterwards mounted using computer-imaging software. This enabled us to show fluorescent activity and its location relative to other wing structures. Second, pictures were taken separately in each of the three bands of wavelengths using a video camera (Sony 3CCD camera DXC-950P) and mounted together using computer-imaging software as semi-transparent pictures. This enabled us to show the location of fluorescent regions having different wavelength sensitivities in one picture.

3. RESULTS

(a) Scarabaeidae

(i) Wing morphology

P. marginata, as all Scarabaeidae, follows the venation and folding pattern of Coleoptera. Differences occur in that the radius anterior (RA) is flexible and has a bending zone and a marginal joint ('Biegungszone' and 'Randgelenk' by Schneider 1978) at about half the wing length (figure 1). The media posterior (MP) 1 + 2 ends at this point. Radius posterior (RP) 3 + 4 forms an articulation with the MP1 + 2 where the transverse fold (tf) crosses. The tf runs further anteriorly and reaches the anterior wing margin at the marginal joint. The longitudinal fold (lf) starts at the medial bridge and branches near the intersection with the tf. The anterior branch ends apically anterior to the RP2. The posterior branch follows RP3 + 4 and MP1 + 2 to the wing margin. The lf with its anterior branch is identical to the median flexion line.

(ii) Resilin occurrence

Fluorescent microscopy in the UV band revealed the presence of resilin in mobile joints of wing veins (figure 2). Resilin is also found at locations with extra mobility, such as the marginal joint and posterior margin of RA (figure 1) and close to the medial bridge, about which RA and MP1 + 2 rotate during folding.

(iii) Wing folding and kinematics

In the folded wing, the anterior wing margin is sharply bent medially (about 100°) at the marginal joint, so that the wing tip comes to lie under cubitus anterior (CuA). The anal area tucks under along the anal fold when the wing is folded. Wing folding is achieved by anteriorly directed brushing movements of the hairy abdominal tergites. This may take several seconds and is not always achieved at the first attempt (F. Hass, personal observation). During folding, RA and MP1 + 2 approach each other, so that they lie almost parallel. When unfolding, the bars separate about 15°, thus unfolding the wing tip (figure 3). The wing of *P. marginata* has a folding ratio (Haas & Wootton 1996) of about 2.3.

Wings of Coleoptera beat with a high amplitude and at high frequencies (95°, 105 Hz, for *Pachnoda sinuata*, Schneider 1997). During flight, there is considerable torsion of the wing. The wing is uniformly bent along its longitudinal axis, showing no indication that the bending zone and marginal joint are exceptionally stiff or soft (figure 4). The wing is also flexed along the median flexion line and the claval fold is visible only in some frames. However, it was not possible to determine the phase in the wing stroke when these folds are active, because of the low temporal resolution.

(b) Coccinellidae

(i) Wing morphology

The venation is reduced in line with the small size of these beetles, if compared to *P. marginata*. In the apical field, the anterior margin is not supported by veins (figure 5). Bending zone and marginal joint are absent and the wing has two transverse folds. The proximal one, the first transverse fold (tf1), crosses the wing at the distal ends of RA and MP1 + 2, whereas the distal one, the second transverse fold (tf2), does not correspond to a vein. The lf begins at the medial bridge, ends at the intersection with tf2 and does not reach the wing margin. The median flexion line (mf) lies posterior to
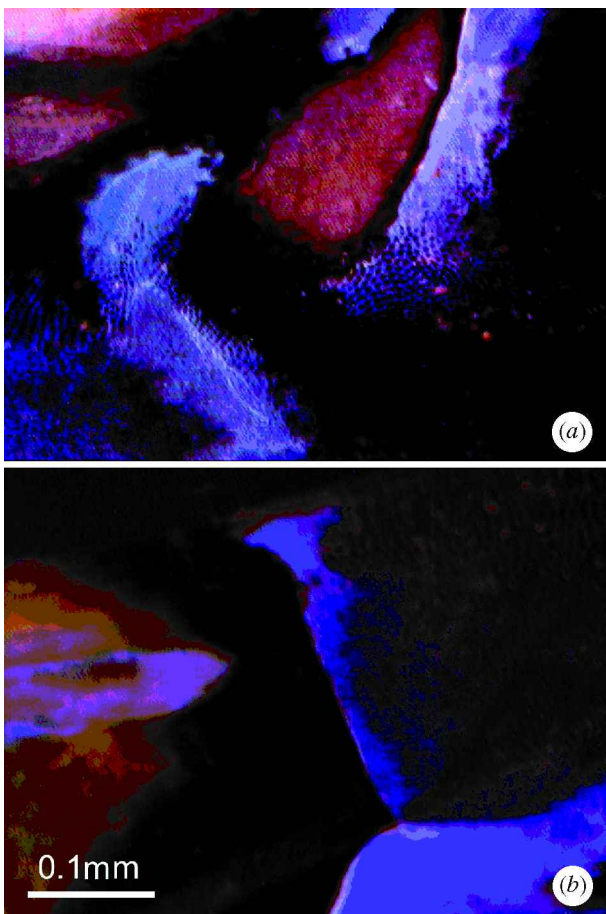
Figure 6. *Coccinella septempunctata*, vein joints supplemented with resilin (fluorescent microscopy). Each picture was made from three pictures taken at different wavelengths and combined. Blue to violet areas contain resilin. For location of the regions in the wing see figure 5. (*a*) Region located anterio-distal from the radius posterior (RP) vein, (*b*) costal wing margin, distal region of the radius anterior (RA) vein.

the lf and follows in its apical half the course of the RP2. In contrast to the wings of most other Coleoptera, there is a stiffening fold (sf) located anterior to the lf and crossing both transverse folds (tf1, tf2).

(ii) *Resilin occurrence*

Fluorescence microscopy in the UV band revealed the presence of resilin in mobile joints of wing veins (figure 6). Resilin is also found at locations with extra mobility, such as the posterior margin of RA and the medial bridge, about which the veins rotate during folding. In contrast to *P. marginata*, resilin occurs in relatively larger areas of the wing and follows the sf and mf to the wing margin.

(iii) *Wing folding and kinematics*

As in *P. marginata*, the coccinellid wing is folded by brushing movements of the hairy abdominal tergites (F. Hass, personal observation). Thus, the RA and MP1 + 2 are approached, and the two transverse folds yield a zigzag pattern in lateral view. Consequently, the wing tip lies caudally in the folded wing. The anal area is tucked under the main wing area along the anal fold (af). During unfolding, RA and MP1 + 2 separate and as a result the wing unfolds to tf2. The wing tip distal to tf2 is unfolded by some unknown mechanism. The wing has a folding ratio of about 2.8.

No video sequences are available for wing torsion during flight. However, still photographs clearly show the presence of the mf, claval fold and sf in the apical wing part during flight (figure 7). These folds, except for sf, are also visible in video sequences of *P. marginata*.

4. DISCUSSION

Resilin, a protein that has been demonstrated to be capable of elastic energy storage (Andersen & Weis-Fogh
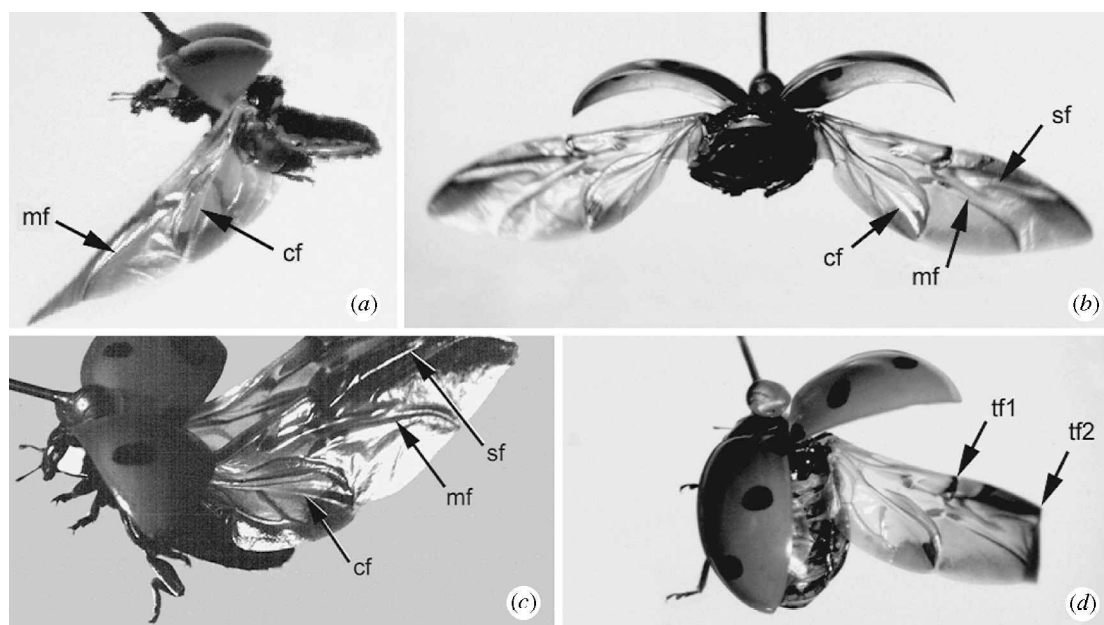

Figure 7. *Coccinella septempunctata*, photographs of flight (*a–c*) and wing unfolding (*d*). (*a,c*) lateral aspect, (*b*) posterior aspect and (*d*) dorsal aspect. cf, claval fold; mf, median flexion line; sf, stiffening fold; tf1, first transverse fold; tf2, second transverse fold.

1964; Rothschild *et al.* 1975; Bennet-Clark 1976), has also been described from jumping systems of Coleoptera (Furth *et al.* 1983; Furth & Suzuki 1992), Siphonaptera (Rothschild *et al.* 1975) and Homoptera (Gorb 1997). It has also been reported from the abdominal cuticle of workers of the honey-ants (Varman 1981) and queen termites (Raghu-Varman 1980), the eye-lens cuticle of the dragonfly *Mesogomphus lineatus* (Viswanathan & Varadaraj 1985), the food pump of reduviid bugs (Edwards 1983), and the tendons of the dragonfly flight muscles (Andersen & Weis-Fogh 1964).

The present paper describes for the first time, to our knowledge, resilin providing elasticity in particular areas of the coleopteran hind wings. In contrast to the closely related Megaloptera, Raphidioptera and Neuroptera, the venation of coleopteran hind wings appears irregular and reduced to two major veins (RA and MP1 + 2), and also the hind wings are folded. Compared to other insects' wings, which are adapted only to flight, the coleopteran hind wings serve another function: folding.

This additional function is reflected in the wing structure, which has folds, bending zones and a marginal joint not found in any other taxon. Furthermore, RA and MP1 + 2 are movable in a scissor-like manner (Forbes 1924; Kukalová-Peck & Lawrence 1993) with the medial bridge serving as pivot. The resilin distribution follows this structure. Resilin is found at those locations where extra flexibility is needed, such as the marginal joint of *P. marginata*. The higher breaking strain (as compared to the surrounding cuticle) of resilin probably prevents material fatigue at these locations. Thus, the wing can be unfolded and folded many times without showing the effects of material fatigue—often visible in the posterior wing margin, which is often frayed in older insects. However, resilin is also found between the veins separating during folding: RA, MP1 + 2 and CuA. This suggests energy storage, because the veins are approximated during folding by the brushing movements of the abdominal tergites (Kaufmann 1960; Blum 1979; Hammond 1979). There, the resilin is deformed between the veins and elastic energy is stored. This energy is released when the wing unfolds, thus supporting the scissor-like movement of RA and MP1 + 2. The stored energy is sufficient to unfold the wings completely when manipulated (Hammond 1979; Brackenbury 1994). However, there is evidence that in the natural situation the stored energy is only supportive and that the unfolding is achieved by the scissor-like movement of RA and MP1 + 2 (Haas 1999).

The presence of resilin in the hind wings of Coleoptera also suggests some function during flight. Some information is available on the flight kinematics and wing mechanics of insects (see Brodsky (1994) and Grodnitsky (1999) for a review). Lift and angle of attack are automatically adjusted by a combination of muscle forces and aerodynamic loading. Consequently, structure and materials of a wing have a great influence on flight performance. Wootton (1981) has given several examples of how the structure of a wing determines its deformation during aerodynamic loading. Our results extend these findings to the material level of the wing, of which only limited information is available. Kesel (1998) suggests the presence of a preferably orientated fibrous network in odonatan wings, responsible

for an asymmetrical resistance of wing cells during loading. However, no chitin has been found in the wing membrane (Richards 1951). We assume that the wing deformation during flight (figures 3 and 4) results in elastic energy storage in wing regions located distal to the articulation. Thus, deformed wings return to an initial position instantly by loaded resilin springs. Elastic elements, such as resilin, probably constitute an essential adaptation for the flapping flight of insects. This possible function of resilin should be experimentally tested or simulated, which was beyond the scope of this paper.

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