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Arthropod Structure & Development 29 (2000) 129–135

ARTHROPOD STRUCTURE & DEVELOPMENT

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Microsculpture of the wing surface in Odonata: evidence for cuticular wax covering

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Received 27 March 2000; accepted 29 May 2000

Abstract

The insect wing membrane is usually covered by scales, hairs, and acanthae, which serve diverse functions, such as species-specific coloration pattern, decrease of wind resistance during flight or decrease of wing wettability. Representatives of Palaeoptera (Odonata and Ephemeroptera) have no hairy structures on the wing membrane, but both its sides are fine-sculptured. In this study, the nature of the wing covering was studied using acoustic microscopy, scanning- and transmission electron microscopy followed by a variety of chemical treatments. It was shown that wing microsculptures are not cuticular outgrowths, but a wax covering, which is similar to pruinosity, which has been previously described in several odonate taxa. Data from scanning acoustic microscopy revealed that scratches on the wax covering have material density different from the surrounding material. Various functions of the wax covering are discussed.

Keywords: Wing; Insecta; Cuticle; Odonata; Microsculpture; Flight

1. Introduction

An insect wing consists of a three-dimensional skeletal network of relatively stiff veins, which are interconnected through thin membranous areas called cells. Dragonflies and damselflies have archaic wing venation (Martynov, 1924; Newman, 1982; Pfau, 1986) and fascinating flight performances, which have been studied in detail (Rüppell, 1989; Wootton, 1991; Wakeling and Ellington, 1997a,b,c). Wings consist of cuticle, a biological fibrous composite material (Neville, 1993), whose mechanical properties may range from very stiff to flexible, depending on its chemical composition (Hepburn and Ball, 1973; Hepburn and Chandler, 1975, 1976; Hackman and Goldberg, 1987). Additionally, vein microjoints of damselflies contain resilin, a rubber-like protein (Andersen and Weis-Fogh, 1964), presumably involved in mechanical control of wing torsion and elastic energy storage (Gorb, 1999).

The cuticle design of wing cells remained unknown until recently. It has been presumed that the membranous cuticle contains a preferentially orientated fibre network responsible for an anisotropic resistance of cells under mechanical loads (Kesel, 1998). The surface of the membranous cuticle is smooth, when viewed in a light microscope and in scanning electron microscopy (SEM) at low magnifications. In reality, the surface has a fine pattern previously described as an irregular terry cloth-like lightly erodable microsculpture (Wagner et al., 1996). Detailed data on the nature and distribution of materials involved in wing design are essential for understanding insect flight aerodynamics and evolution of insect wings. This study has been undertaken to clarify the nature of the fibrous network and covering of wing membranes. Since such small cuticle outgrowths have not been previously reported, we presumed that wing microsculpture is due to the wax covering.

2. Material and methods

2.1. Animals

Wings of freshly killed and dried damselflies *Pyrrhosoma nymphula* and *Coenagrion puella* (Zygoptera, Coenagrionidae) were used for transmission electron microscopy (TEM) and SEM. Wings of freshly killed dragonflies *Aeshna cyanea* (Anisoptera, Aeshnidae) were used for the scanning acoustic microscopy (SAM). A variety of methods was used to exclude artefacts, which may appear when only one method is used.

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1467-8039/00/$ - see front matter
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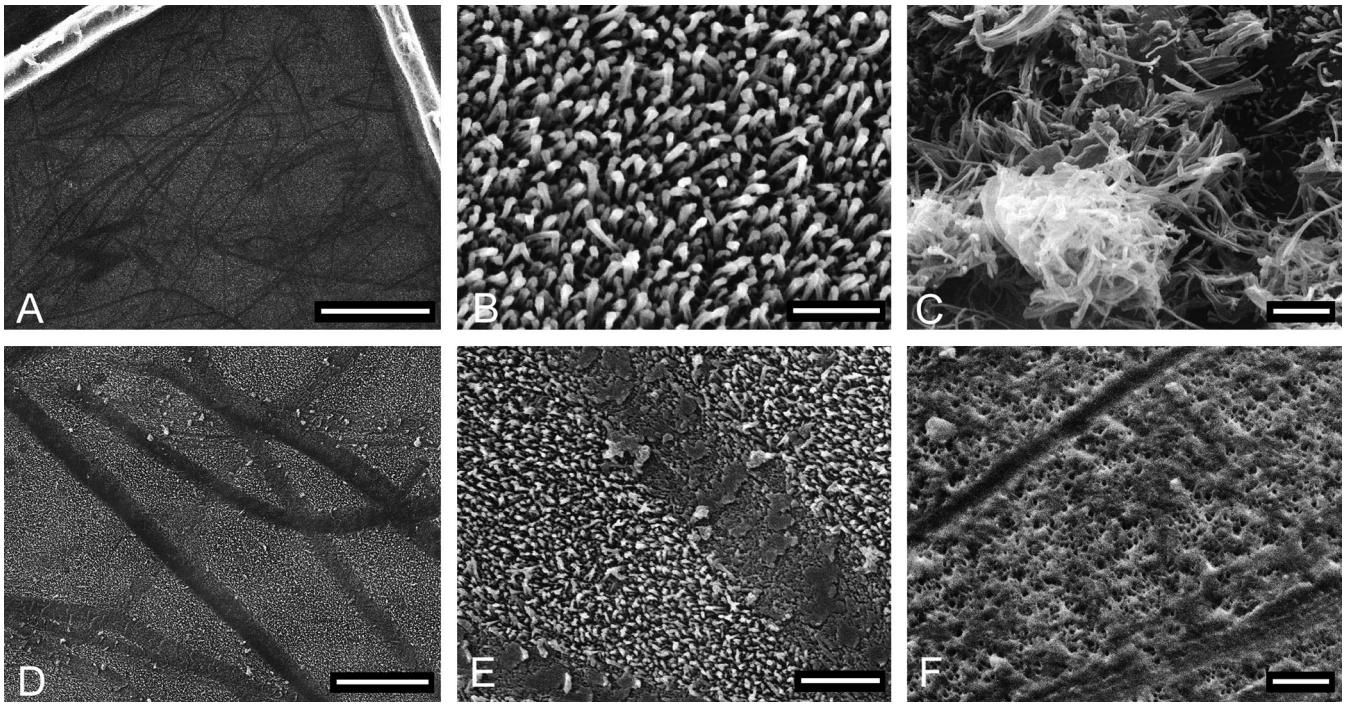


Fig. 1. Scanning electron microscopy of intact wings in: (A) and (F), *P. nymphula*; and (B–E), *C. puella*. (A) The overview of the wing membrane with two veins (scale bar = 50 μm). (B) Microsculpture of the wing membrane (scale bar = 0.5 μm). (C) Microsculpture of the vein surface (scale bar = 1 μm. (D)–(F) Scratches of the membrane. (A)–(E) Intact wings coated with gold–palladium. (F) Intact wing coated with carbon–platinum. (D) Scale bar = 10 μm. (E) Scale bar = 2 μm. (F) Scale bar = 1 μm.

2.2. Scanning and transmission electron microscopy

2.2.1. Control

Wings were cut off, mounted on holders, sputter-coated with gold–palladium (10 nm) and examined in a SEM Hitachi S-800 at 20 kV.

2.2.2. Treatment in organic solvents

Wing parts were treated for 1 h in a variety of solvents, such as absolute ethanol, absolute methanol, absolute acetone, 1,2-propylenoxide, 1,2-dichlorethane, heptane, and chloroform, mounted on holders, sputter-coated with gold–palladium and studied in the SEM.

2.2.3. TEM of air-dried wings

Wing parts were mounted in oyster-grids (Plano, Germany) and examined in TEM Philips CM10.

2.2.4. TEM of ultrathin sections

Freshly cut pieces of wings (1–1.5 mm) were fixed for 48 h at 4°C in 2.5% glutaraldehyde (in 0.01 M phosphate buffer at pH 7.3), and postfixed for 1 h in 1% osmium tetroxide in phosphate buffer at 2°C. After washing, preparations were stained for 1 h at 4°C in 0.1% aqueous uranyl acetate solution, washed, dehydrated and embedded in a low-viscosity resin (Spurr, 1969). Ultrathin sections were prepared from Spurr-embedded blocks and picked up on copper grids coated with pioloform film. Sections were stained with uranyl acetate and lead citrate, and studied in a TEM.

2.2.5. TEM and SEM of wings coated with carbon–platinum

Wings mounted in the oyster-grids were coated with carbon–platinum at an angle of about 15° to contrast surface relief and examined in SEM or TEM.

2.3. Acoustic microscopy

A scanning acoustic microscope (KSI SAM 2000) was used to obtain qualitative information on the mechanical characteristics of the wing membranes. Reflections of the ultrasonic waves provide information on both the surface topography and the material characteristics, e.g. the material density. In order to analyse the vein-framed membranes, they were mounted on holders and covered with distilled water (25°C). The ultrasonic frequency was 200 MHz.

3. Results

3.1. Structure of the wing covering

SEM revealed the presence of a fine dust-like covering on both sides of the wing membrane. The surface also contains randomly oriented stripes resembling scratches on the surface (Fig. 1A and D). The width of the scratches varies from 0.94 to 4.01 μm (mean = 1.81, s.d. = 0.63, $n = 42$).

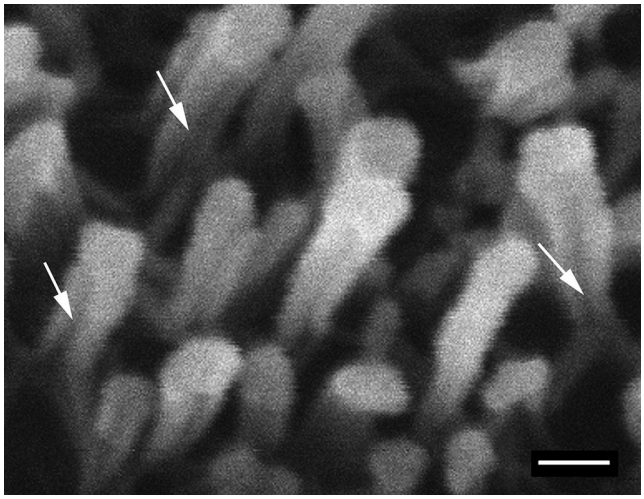


Fig. 2. *C. puella*, microsculpture of the wing membrane (scale bar = 100 nm). Arrows indicate pairs of joined structures.

At a higher magnification, the wing membrane covering resembles tiny rods (length = 290 nm, s.d. = 40, $n = 23$; width = 60 nm, s.d. = 10, $n = 36$) (Fig. 1B). The density of these structures is 69.0 per 1 μm^2 (s.d. = 4.1, $n = 5$). The surface of the scratches also appeared rough (Fig. 1E). In carbon–platinum-coated preparations, the scratches appeared as depressions under the level of the covering (Fig. 1F). The depressions contain compressed rod material. Lengths of the rods measured in carbon–platinum-coated preparations were 0.42 μm (s.d. = 0.10, $n = 10$). Lengths of the compressed rods measured in scratches were 0.22 μm (s.d. = 0.02, $n = 8$). The rod is often composed of two subunits (Fig. 2). The rod structures are interconnected at their bases via net-like structures, which are easily viewed in uncoated wings in TEM (Fig. 3C and D). In the carbon–platinum-coated wings viewed in TEM, rods appeared as a structured covering (Fig. 3G), which was often partly removed from the surface (Fig. 3H).

The wing veins were also covered by rods, which were much longer and variable, compared with those of the wing membrane (length = 970 nm, s.d. = 310, $n = 40$). However, these structures have a width comparable to those occurring on the wing membrane (width = 40 nm, s.d. = 10, $n = 40$) (Fig. 1C).

3.2. Ultrastructure of the cuticle of the wing membrane

The wing membrane is about 2 μm thick and mainly composed of cuticle consisting of lamellae of about 25 nm thickness (Fig. 3A and B). This lamellate cuticle is presumably procuticle; however, the presence of chitin was not tested. The lamellate cuticle is covered by epicuticle (20–30 nm thick), which is perfectly smooth. This means that the rod structures described in previous sections have extracuticular origin. In ultrathin sections, these structures were usually not visible (Fig. 3B) or appeared as electron-lucent remains of material (Fig. 3A). There are no epidermal cells in the membrane; however, pore canals often occurred.

3.3. Effect of solvents on the membrane covering

A destructive effect of organic solvents on the membrane covering occurred in ultrathin sections under TEM. Since microsculpture of the structures covering the wing membrane are somewhat different from the usual odonate pruinescence, we decided to test the covering of the wing membrane in a variety of solvents. The results of these experiments are shown in Fig. 4. As a reference, an example of an intact surface is given in Fig. 4L.

All the solvents used had a destructive effect on the covering. Heptane- (Fig. 4A and B), methanol- (Fig. 4C) and ethanol-treated surfaces (Fig. 4D and E) looked as if they were covered with an amorphous material; however, some areas looked relatively intact. There were several areas (Fig. 4A and E), where the covering was completely removed. In the light microscope, chloroform-treated wing surfaces did not appear smoky as intact wings usually do, but appeared absolutely transparent. SEM preparations have revealed that the wing covering was completely dissolved. Some remaining material was found only in some areas of the wing (Fig. 4F). Dichlorethane-treated surfaces contained both absolutely intact areas as well as amorphously covered areas (Fig. 4G and H). In wings treated with propylene-oxide, the rods looked quite damaged, and some wing areas completely lacked wax covering (Fig. 4I). In acetone-treated wings, the covering was still present, but usually appeared strongly covered with an amorphous material (Fig. 4J and K).

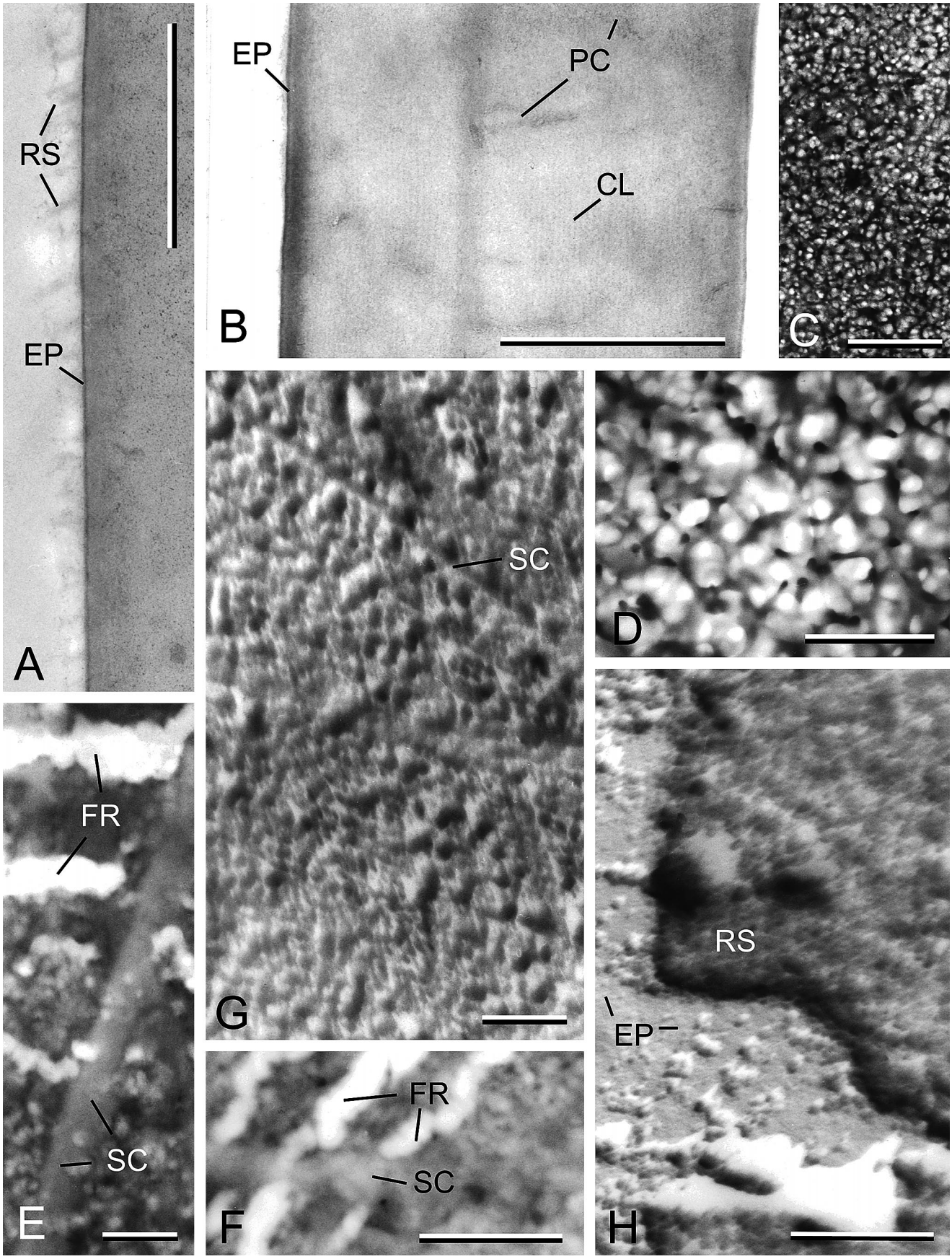
3.4. Relative material density

SAM data on material design of the wing membrane show that the membrane contains a preferentially orientated fibre network (Fig. 5). The dimensions of the fibres approximately correspond to the scratches obtained from the SEM and TEM preparations. SAM measurements revealed that the density of fibre-like structures differs from that of the surrounding material. No fibre-like pattern was found in the SAM measurements of membranes without the waxy layer.

4. Discussion

4.1. Nature of the odonate wing covering

Vein covering resembles the filamentous pruinosity previously described from axillary sclerites and the antero-dorsal surface of the pterothorax in two odonate species, *Bayadera indica* (Euphaeidae) and *Mnais pruinosa* (Calopterygidae) (Gorb, 1995). Filamentous pruinosity is a kind of structural coloration, which is actually a layer of wax crystals which compounds are secreted in epidermal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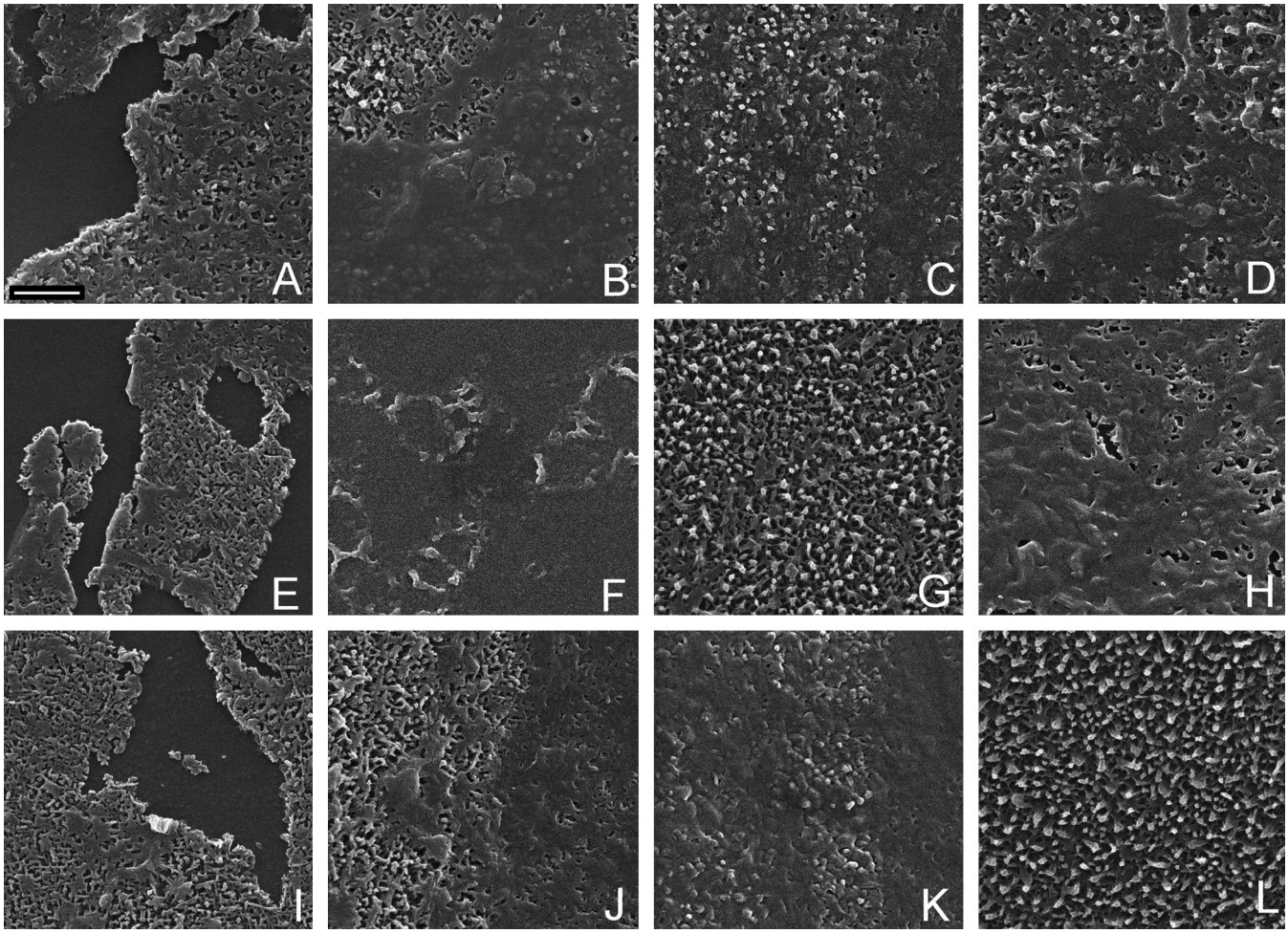

Fig. 4. *C. puella*, results of experiments with solvent treatments, scanning electron microscopy. (A) and (B), heptane. (C) Absolute methanol. (D) and (E), absolute ethanol. (F) Chloroform. (G) and (H), 1,2-dichlorethane. (I) 1,2-Propylenoxide. (J) and (K), absolute acetone. (L) Intact wing. Scale = 1 μm.

cells and transported to the cuticle surface by a system of pore canals (Gorb, 1997).

Experiments with organic solvent treatments additionally support the idea that structures occurring on the surface of the wing membrane are wax-like crystals, which material is presumably transported to the surface from wing epidermal cells during or shortly after emergence. In adult Odonata, epidermal cells secreting the wing membrane are absent and the number of pore canals is strongly reduced. This means that the wax covering is secreted once in the life-time of the insect and neither renewed nor added to thereafter. Therefore, the whole pattern of scratches reflects the individual history of an insect: as a rule, the number of scratches was greater in older individuals compared with younger ones.

4.2. Possible functions of the covering

Similar structures were observed in representatives of Ephemeroptera, which are closely related to Odonata (Wagner et al., 1996; this study) and Planipennia (Wagner et al., 1996). One possible function of the wax covering is to decrease wettability of wings, which can be essential for insects closely connected, in their life-history, with water-bodies. Odonata females lay their eggs in water or in the vicinity of water, whereas males often defend their territories over water. Moreover, the danger of falling into water is even higher in freshly emerged individuals. Some damselfly females even submerge in water to lay their eggs (Fincke, 1986). Due to the wax covering, the wings remain non-wetted, holding a reasonable amount of air. The

Fig. 3. *P. nymphula*, transmission electron microscopy of the wing membrane. (A) and (B), ultrathin section of the wing membrane. (C) and (D), wing membrane viewed in TEM. (E) and (F), scratches and fractures in the wing membrane. (G) and (H), wing membrane coated with carbon–platinum. (C) and (F)–(H), scale bars = 2 μm. (A), (B), (D) and (E), scale bars = 1 μm. CL, cuticle layers; EP, epicuticle; FR, fractures; PC, pore canals; RS, rest of the surface structure; and SC, scratches.

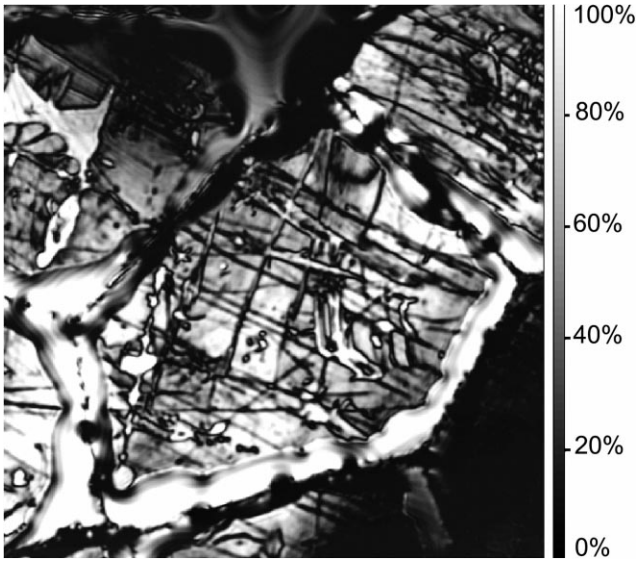

Fig. 5. *Aeshna cyanea*, SAM of a vein-framed dragonfly wing membrane. Fibre-like structures running crosswise in the wing membranes. Different material densities are represented by variations in the grey shading.

wax-like wing covering can also prevent wing contamination as has been shown by Wagner et al. (1996).

Knowing that the odonate wax covering located on the thorax or abdomen can reflect ultraviolet light (Robertson, 1984; Hilton, 1986) and that dragonflies can recognise a UV coloration pattern (Frantsevich and Mokrushov, 1984), we may suggest that wax covering of wings can play some role in intra- and inter-specific communication. Indeed, it has been previously shown that *Lestes* and *Coenagrion* damselfly males, which usually can easily recognise dead females with partly removed body parts, do not recognise females with removed wings (Mokrushov, 1992; Gorb, 1998). Pruinescence reflecting UV in odonate males probably aids thermoregulation by reflecting radiation (Paulson, 1983; Ubukata, 1985). The same function also can be suggested for the wax covering of wings.

Overall thickness of the wing covering is about 0.6 μm, which is about one-third of the cuticle thickness of the wing membrane. It can be suggested that the mass of the wax covering can influence wing mechanics. Additionally, the surface covered with wax crystals becomes rough. Rough surfaces of flying insects, birds and fish serve to reduce air or water resistance due to microturbulence (Nachtigall et al., 1998). However, the wax roughness of damselfly wings is 1–2 magnitudes smaller compared with the roughness responsible for air microturbulence in animals with comparable Reynolds numbers. It seems that wax roughness cannot contribute to “the shark-skin effect” (Nachtigall et al., 1998).

The wax covering seems to be mechanically more stable in areas of scratches. Fractures of the membrane covering usually do not cross scratches (Fig. 3E and F). Thus, the compressed material of scratches may influence the mechanical properties of the wing membrane. Data from acoustic microscopy revealed a preferentially orientated fibre network, which is presumably responsible for an asymmetric resistance of cells under mechanical loading. The fact that no fibre-like pattern was found in the SAM measurements of membranes without the waxy layer led us to suggest that the fibre-like pattern is a part of the covering layer. Thus, the SAM measurements could be interpreted as an additional indication of the mechanical characteristic of the wax covering, modified by the scratches. The increased stiffness affects the mechanical stability of the membranes and thus the stability of the whole wing system, as found by means of numerical analyses (FEM) (Kesel et al., 1998).

Acknowledgements

Our sincere thanks to Prof. Dr U. Schwarz (MPI für Enwicklungsbiologie, Tübingen, Germany) for discussions about structure and functions of biomaterials, Prof. Dr W. Arnold (Fraunhofer-Institut für zerstörungsfreie Prüfverfahren, Saarbrücken), and Dipl.-Biol. P. Kreuz (Institut für Zoologie, Universität Saarbrücken) for discussions and help with the SAM measurements. Dr H. Schwarz (MPI für Enwicklungsbiologie, Tübingen, Germany) helped with TEM procedures. V. Kastner has made valuable linguistic corrections on the early version of the manuscript. This study was partly supported by the Federal Ministry of Science of Germany (BMBF) grant BioFuture 0311851 to S.G.

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