Chapter 7
Brochosomal Coatings of the Integument of Leafhoppers (Hemiptera, Cicadellidae)

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7.1 Introduction

Superhydrophobic properties of natural and man-made surfaces arise from the combination of chemical hydrophobicity with complex, fractal texture at the micron and submicron range (reviewed in Quéré 2005). In plants and animals alike, such complex textures are usually formed by outgrowths of the integument or by particles of wax secreted by the integument (Cassie and Baxter 1945, Fogg 1948, Holdgate 1955, Holloway 1970, Juniper 1991, Wagner et al. 1996, 2003, Barthlott and Neinhuis 1997, Feng et al. 2002). In most cases, the location of these textures on the body and their regular structure are passively determined by the underlying epidermis. Some insects, however, produce and maintain particulate hydrophobic coatings on their bodies in specific behaviors. Whiteflies (Hemiptera: Aleyrodidae) and dustywings (Neuroptera: Coniopterygidae) use their legs to actively distribute wax particles, produced by specialized epidermal glands in certain areas of the body, over the rest of the integument (Navone 1987, Byrne and Hadley 1988, Nelson et al. 2003). In both of these taxa the geometry of the wax particles is remarkably complex, which may enhance the hydrophobic properties of their layers. Apparently, the production of such complex products requires greater specialization and, consequently, more restricted localization of the secretory epidermis, which, in turn, makes necessary their active distribution. These insects may also benefit from being able to modify the coating at will, for example, to quickly fix damage.

Leafhoppers (Hemiptera: Cicadellidae) also coat their integument actively, but with a completely different material: protein-lipid granules produced by the Malpighian tubules and released after molts through the hindgut. Usually, these granules are hollow microspheres 0.2–0.7 μm in diameter with a honeycombed surface (Fig. 7.1A–F), which is reflected in their name, *brochosomes* (BS), derived from the Greek words brochos, “mesh of a net”, and soma, “body” (Tulloch and

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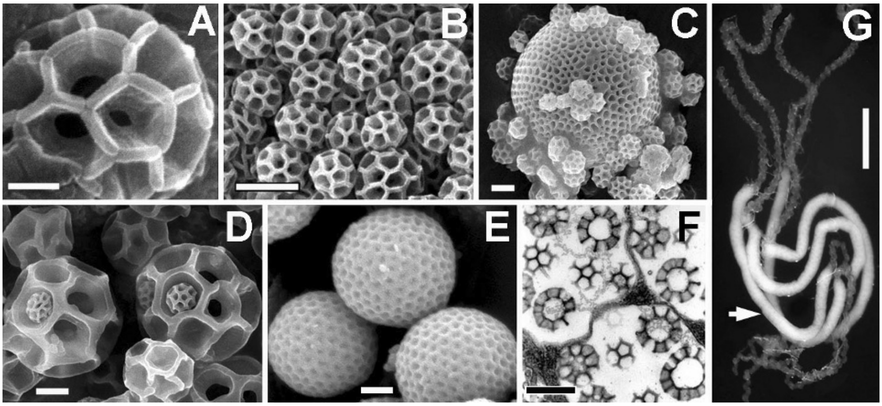

Fig. 7.1 IBS and their genesis in the Malpighian tubules. **A**. A scanning electron micrograph of an intact IBS of *Draeculacephala* sp. **B**. Same, IBS of *Paraphlepsius irroratus* (Say) washed off the integument with acetone (see Section 7.4.2 for details). **C**. Same, intact IBS of *Proconia esmeraldae* Melichar. **D**. Same, *Diestostemma stesilea* Distant. **E**. Same, *Xestocephalus desertorum* (Berg), a nymph. **F**. A transmission electron micrograph of a Malpighian tubule secretory cell of *Igutettix oculata* (Lindberg) showing three vacuoles with mature IBS. **G**. The Malpighian tubules of male *Cuerna yuccae* Oman and Beamer (*arrow*: glandular segments). Scale bars: A, 0.1 μm; B–F, 0.5 μm; G, 1 mm

Shapiro 1953). The properties and the exact adaptive significance of this unique biomaterial remain poorly understood. Several authors observed that layers of BS are highly water-repellent (Günthart 1977, Arzone 1986, Navone 1987, Rakitov 1995, 2002a). In fact, the geometry of these layers is remarkably similar to that of the artificial superhydrophobic surfaces recently created by nanotechnologists (Ming et al. 2005). It has been hypothesized that the BS coatings protect leafhoppers from water and their own liquid excrement. Yet, because the integument of most insects, including leafhoppers, is capable of producing extracuticular waxes, which are thought to serve similar functions, the evolutionary origin of BS remains a puzzle. This motivated researchers to advance alternative hypotheses about the function of BS (reviewed in Rakitov 2002a).

The goal of this chapter is to summarize the available data regarding the origin, form, and function of the BS coatings of leafhoppers to set the stage for further study of these unusual biological surfaces. The literature review is supplemented with previously unpublished data on the amino acid composition of BS.

7.2 Biology of Leafhoppers and Occurrence and Functional Types of BS

With over 23,000 species described, leafhoppers represent the largest radiation of extant Hemiptera. These are jumping insects, the majority between 3 and 8 mm in length, which feed via piercing-sucking mouthparts on plant sap and occur everywhere the vascular plants grow, including wetlands, deserts, and tropical forest canopies. Most leafhopper species coat their integument with BS (Rakitov 1995, 1998, 2002a). A few known exceptions appear to be secondary losses (Rakitov 1998). The BS coatings have been found in all the subfamilies and major tribes of Cicadellidae. In some of these lineages, BS are produced and applied to the integument in both immatures and adults, while in others the production of BS begins during the last nymphal instar, so that BS are released and transferred onto the integument only after the final molt. In the last case, the Malpighian tubules of the young immatures manufacture different, particulate or non-particulate products (Rakitov 1999), which are applied onto the nymphal integument after molts, but dry without forming dense deposits or hydrophobic layers (Rakitov 1996). Their function remains enigmatic.

Females from 18 genera from the Neotropical leafhopper tribes Proconiini and Phereurhinini use BS also to powder their eggs and oviposition sites. In most species from this group, females secrete a specialized type of particles, *egg brochosome*s (EBS), which are morphologically distinct from the common *integumental brochosomes* (IBS). EBS have been recently described in detail (Rakitov 2004) and will be only briefly discussed below in Part 6. Most of the following text focuses on IBS.

Production of BS is unique to Cicadellidae. In the closely related family Membracidae similar behaviors and glandular specializations of the Malpighian tubules occur (Rakitov 1996), but BS have not been found (Dietrich 1989, Deitz and Dietrich 1993). IBS readily contaminate foreign objects, such as entomological collecting equipment: aspirators, forceps, and nets. This explains reports of IBS found on the cuticle of other insect taxa (e.g., Tulloch et al. 1952). IBS have also been found in samples of atmospheric aerosols (Neville and Smith 1970, Bigg 2003, Wittmaack 2005).

7.3 Integumental Brochosomes (IBS)

7.3.1 Structure and Diversity of IBS

IBS of all studied leafhopper species are hollow spheres. Their size and structure can vary among species, developmental stages, sexes, or individual particles on the same leafhopper (Fig. 7.1A–F). Nevertheless, in the vast majority of studied species IBS are highly similar: 0.2–0.7 μm in diameter, with the honeycomblike wall comprised of penta- and hexagonal cells, each with a hole leading into the internal space (Fig. 7.1A–B). Most publications describe exclusively this type of IBS. IBS of this type often, although not always, display the symmetry of the truncate dodecahedron with 20 hexagonal and 12 pentagonal faces, also found in the soccer ball and the C_{60} buckminsterfullerene molecule. Larger IBS, up to 5.0 μm in diameter, occur more rarely, but their structure is more diverse (Fig. 7.1C–E, see also Rakitov 1995, 1999). The smallest average size of IBS has been recorded in *Idiocerus stigmaticalis* Lewis (all particles on the integument between 0.2–0.3 μm), and the largest (3.0–4.8 μm) in *Haranga* sp. (Rakitov 1995). Some leafhoppers coat their body with a mix of two different types of IBS (Fig. 7.1C–D) or with a broad continuum of diverse particles. In *Xestocephalus desertorum* (Berg), IBS are 0.7–2.0 μm in diameter in the immatures (Fig. 7.1E) and 0.3–0.4 μm in the adults (not shown, see Rakitov 2000a). In *Proconia esmeraldae* Melichar, males and females produce different IBS (Rakitov 2004). There is no overall correlation between the structure of IBS and the taxonomy of leafhoppers: closely related species can produce different and unrelated species identical IBS (Rakitov 1995, 2004).

7.3.2 Genesis of IBS

IBS are produced in specialized glandular segments of the Malpighian tubules (Fig. 7.1G) and are released through the hindgut. The Malpighian tubules are the main excretory organ of insects and are primarily engaged in ion exchange and eliminating wastes. However, these organs can also synthesize various materials for external use, such as cocoon silks in Neuroptera (Lozinski 1911, Akai 1998), certain Coleoptera (Lebedew 1914, Mazzi and Baccetti 1956, Kenchington 1983), and Hymenoptera (Mello 1979). Additional examples are discussed in Part 7 and in Rakitov (2002b). Production of IBS in leafhoppers is one of the most remarkable examples of acquiring a secondary function by these organs.

The glandular segments of the Malpighian tubules of Cicadellidae (Fig. 7.1G, arrow) are comprised of large cells, each simultaneously producing a vast number of IBS (Fig. 7.1F, see also Smith and Littau 1960, Gouranton 1967, Rakitov 1999, 2000b). Unlike other parts of the tubules, the glandular segments lack ultrastructural features associated with excretion. The cytoplasm of their cells displays an extensively developed rough endoplasmic reticulum, as is typical of protein-secreting cells. IBS gradually acquire their shape inside Golgi-derived vacuoles. The surface of the originally globular particles becomes molded with closely-set invaginations, which eventually form the honeycombed surface. Vacuoles with the mature particles (Fig. 7.1F) empty into the tubule lumen.

7.3.3 Anointing and Grooming

The IBS coatings result from two specific behaviors. During *anointing*, which usually occurs within 1–3 hrs after molt, a colloidal suspension of IBS is released through the hindgut and applied onto the integument. After the liquid dries, IBS are further spread over the cuticle during repeated bouts of *grooming* (Storey and Nichols 1937, Navone 1987, Rakitov 1992, 1996, 2000a).

Prior to anointing, freshly molted leafhoppers do not feed and generally remain motionless. Details of anointing vary between species and between life stages. Most nymphs use their legs to smear the fluid over the ventral parts of their bodies (Fig. 7.2A) and then roll their abdomens upward to spread the fluid over the dorsal surfaces (Fig. 7.2B). An adult leafhopper usually picks up a droplet of the secretion from the anus with its hind legs and spreads it all over the ventral body surface and appendages, a few minutes later it releases additional droplets, transfers them onto the forewings, and spreads them over the dorsal side of the forewings, pronotum,

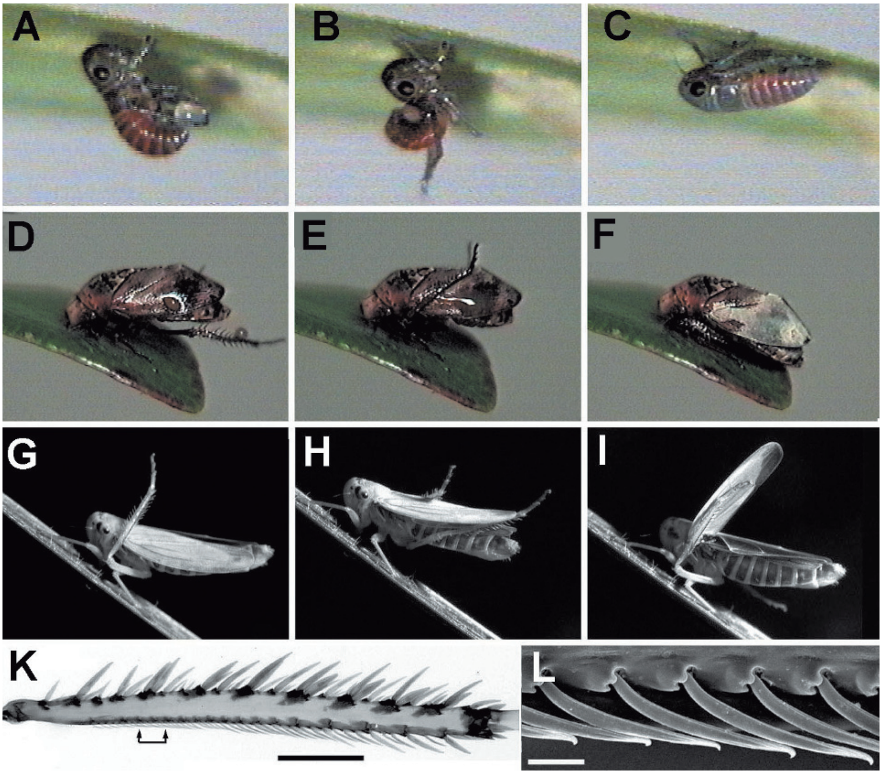

Fig. 7.2 Behaviors and structures involved in producing of the IBS coatings. **A**–**C**. Anointing in a 1st instar nymph of *Cuerna costalis* (F.). **D**–**F**. Anointing in adult *Penthimia americana* Fitch. **G**–**I**. Grooming in adult *Cicadella viridis* (L.), strokes with metathoracic tibiae. **K**. Metathoracic tibia of *Paraphlepsius irroratus*. L. Same, a scanning electron micrograph of the basal setae of the posteroventral row, indicated on the preceding figure with *arrows*. Scale bars: K, 0.5 mm; L, 25 µm

and head (Fig. 7.2D–E). The fluid evaporates leaving a deposit of dry IBS (Fig. 7.2C and F).

During grooming (Fig. 7.2G–I) leafhoppers scrub and brush their bodies and appendages with rapid strokes of the legs (up to 11 Hz – D. Tishechkin, personal communication). While in most insects grooming is a hygienic procedure, in leafhoppers its main function appears to be redistribution of dry IBS over the integument (Navone 1987, Rakitov 1996, 1998). What triggers repeated bouts of grooming and how they modify an existing IBS coating remains unknown. One of their functions can be maintaining the evenness and continuity of the IBS layer.

The relative contributions of anointing and grooming to manufacturing of the IBS coatings vary. In immatures, the secretion can be spread across the entire body surface in the liquid form during anointing (Fig. 7.2A–C). In the winged adults the liquid is spread over the exposed integument (Fig. 7.2D–F), but the hindwings and the dorsal surface of the abdomen, concealed at rest by the forewings, become

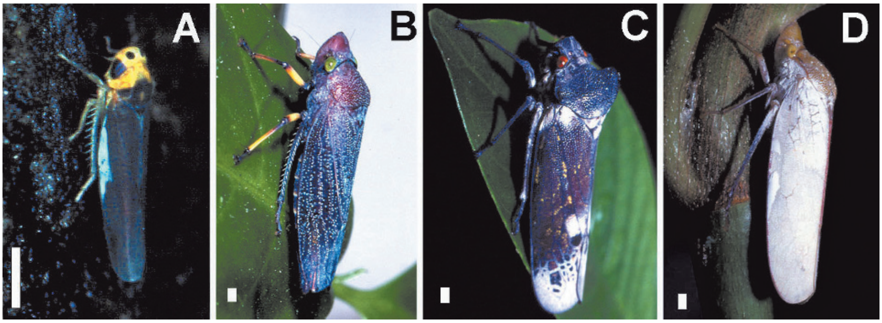

Fig. 7.3 External appearance of the IBS coatings. **A**. *Salka* sp., note the accumulation of pale IBS near the leading edge of the forewing. Photo: **C**. Dietrich. **B**. *Abana gigas* (Fowler). **C**. *Proconia* sp. **D**. *Diestostemma* sp. Scale bars: A–D, 1 mm

coated with IBS during the subsequent grooming. Finally, in the subfamily Typhlocybinae, the adults do not spread droplets of secretion during anointing, but place them onto specialized sculptured areas of the forewings, where the secretion dries as a pair of spots (Fig. 7.3A). The coating of the entire integument is made by distributing the material of these spots during grooming (Vidano and Arzone 1984, Navone 1987, Rakitov 1992, 1996, Humphrey and Dworakowska 2002).

7.3.4 Tools for Manipulating IBS

One of the diagnostic traits of Cicadellidae are well differentiated rows and groups of strong setae on the legs (Fig. 7.2K). These structures act as brushes spreading the liquid secretion or as rakes spreading the dry IBS during anointing and grooming, respectively (Vidano and Arzone 1984, Navone 1987, Rakitov 1998, Humphrey and Dworakowska 2002). The setae can be simple acute or specialized: spatulate, capitate, inflated, or hooked. In particular, in nearly all studied leafhopper species the basal part of the posteroventral setal row on the hind tibia contains numerous small, delicate setae with hooked apices (Fig. 7.2L). Their chief function appears to be gently loosening the crust of the IBS deposited on the dorsal surface of the forewing, so that the material can be transferred onto other areas with minimal losses (Rakitov 1998). A better differentiation of the setal armament on the legs correlates with the presence of thin and uniform coatings of IBS; both species with sparse and those with copious IBS coatings tend to have less specialized legs (op. cit.).

7.3.5 The IBS Coatings

Such coatings (Figs. 7.3A–D, 7.4A–I) vary in continuity and thickness among species, individuals, and body parts of the same individual from scattered particles to copious layers. In the vast majority of leafhoppers these coatings are thin and barely

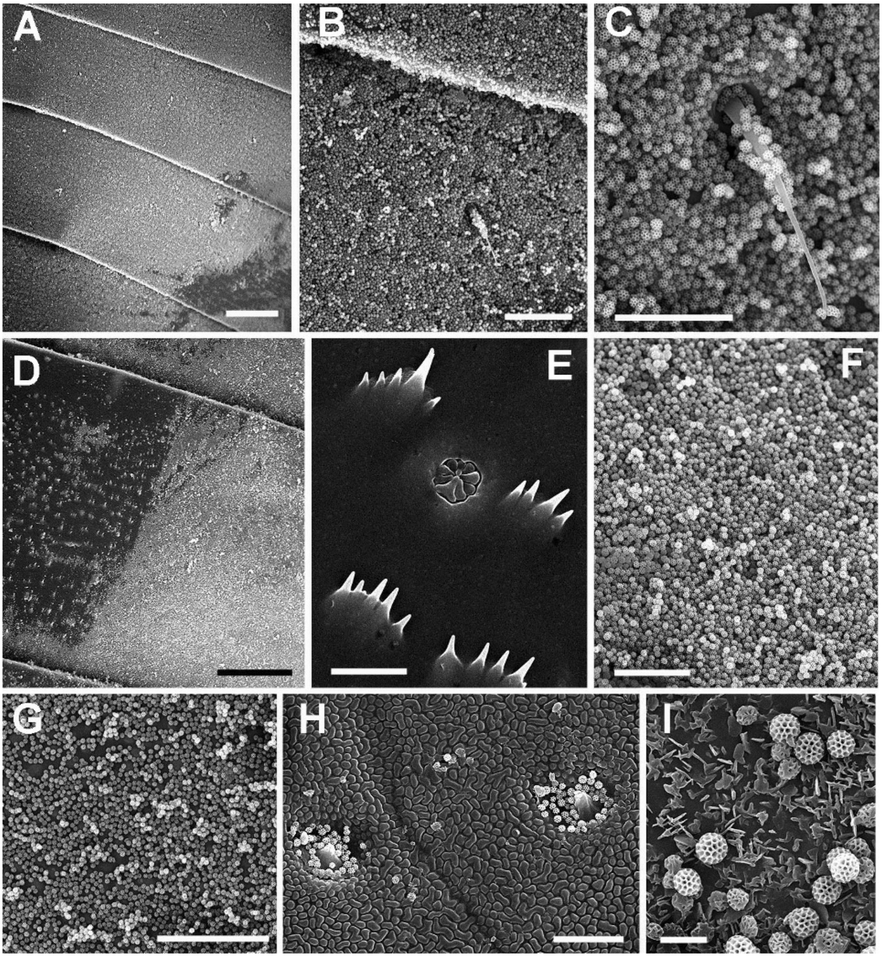

Fig. 7.4 Scanning electron micrographs of the IBS coatings. **A**. *Aceratagallia* sp., abdominal tergites, the coating is damaged in the lower right corner (dark areas). **B**. Same, close-up. **C**. same, close-up of a seta. **D**. *Paraphlepsius irroratus*, an abdominal tergite, on the *left* side the IBS coating has been brushed off making the cuticular sculpture visible. **E**. Same, close-up of the cleaned cuticle showing cuticular protuberances and a coeloconic sensillum. **F**. Same, intact coating of the tergite, shown at the same magnification. **G**. Same, ventral surface of a forewing. **H**. *Onega orphne* Takiya and Cavichioli, dorsal surface of a forewing with two chaetoids or sensilla. **I**. *Helochara communis* Fitch, IBS on a carpet of wax crystals on a hind wing. Scale bars: A, D, 50 μm; B, G,10 μm; C, E, F, H, 5 μm; I, 1 μm

visible in the stereomicroscope. On the darker body parts they can be noticeable as bluish deposits, this color being produced by Tyndall scattering of light on particles <1 μm in diameter (Fig. 7.3A–B). Thicker coats, visible by naked eye, occur more rarely and resemble white waxy blooms of other insects (Fig. 7.3C–D).

The leafhopper cuticle typically lacks a hair coat, but can be sculptured with microscopic protuberances and sensilla (Fig. 7.4D–E, see also Dietrich 1989, Rakitov 1995). A well developed IBS coating can conceal these structures completely (compare Fig. 7.4E and F). IBS can caulk sutures and folds between sclerites; in meager coatings they are often seen accumulated in sensillar pits (Fig. 7.4H) and other microscopic depressions.

Production of extracuticular wax is generally uncharacteristic of Cicadellidae, but small amounts of particulate wax have been found on the hind wings (Fig. 7.4I) and other body parts of several leafhopper species (Rakitov 1995).

7.4 Properties of IBS and Their Layers

7.4.1 Chemical Composition of IBS

Previous studies have used histochemical methods and electron microscopic observations of samples digested with enzymes to demonstrate that IBS contain proteins and phospholipids. One such study suggested that an individual particle consists of a protein skeleton coated with saturated lipids (Smith and Littau 1960), while another argued that the entire particle is formed by a protein-lipid complex (Gouranton and Maillet 1967). Additionally, Gouranton (1967) detected an alkaline phosphatase in developing IBS from the Malpighian tubules of *Cicadella viridis* (L.), but not in two other species examined. Mayse (1981) was able to detect allantoin and urea in EBS of *Oncometopia orbona* (F.), but did not indicate whether these compounds were major constituents in the material. IBS are not generally affected by organic solvents (Gouranton 1967). Therefore, solvents can be used to wash IBS off the leafhopper integument. This approach was employed to prepare samples for a study of the amino acid composition of IBS as described below.

Experiment 1. Amino Acid Analysis of Integumental Brochosomes

The starting material was 4.0 g of intact dry specimens of *Paraphlepsius irroratus* (Say), which amounted to approximately 1,600 insects. The leafhoppers were collected in Urbana, Illinois, at light and killed at −20°C. The sample was placed in 40 ml of acetone and gently shaken for 1 min. The mix was filtered successively through Whatman paper filters #4 and #2 to remove the insect bodies and derbris above 25 μm and 8 μm, respectively. Approximately 20 ml of the filtrate were aliquoted into two polypropylene copolymer centrifuge tubes and centrifuged for 30 min at 1000 g. Then the supernatant was carefully decanted and replaced with 10 ml of fresh acetone in each tube. The tubes were sonicated for 3 min to resuspend pellets and then centrifuged at 2900 g for 15 min, after which the supernatants were replaced and pellets resuspended again. Four additional similar cycles of centrifuging followed by resuspension in fresh acetone were performed at 2900 g (15 min), 11500 g (7 min), 11500 g (7 min), and 16600 g (7 min). After the last cycle each pellet was resuspended in only 2 ml of acetone, the two samples were combined, and then filtered through a syringe filter with 5.0 μm pores into a pre-weighed glass vial. The sample was dried under vacuum. All the above steps were performed at room temperature. The resulting sediment was pure white, non-shiny, homogeneous, and without visible impurities. Its weight was estimated as 1167±20 μg. After the amino acid analysis (see below) the remainder of this sample was examined by SEM to confirm that the sediment consisted of clean IBS, not visibly affected by the treatment (Fig. 7.1B). Therefore, it is reasonable to assume that the broken fragments present in the original acetone mix were retained by the paper filters, the bulk of the soluble compounds in that mix was removed during successive cycles of washing and pelleting, and that no significant fraction of the material that formed the structure of the intact IBS was lost during the sample preparation. Consequently, the amino acid composition of the sample was expected to accurately characterize the protein component of the intact IBS.

Prior to analysis, the entire sample was resuspended in 0.5 ml of acetone and sonicated for 5 min to assure complete and homogeneous dispersion of the particles. Three replicates of the analysis were performed, each assaying a 10 μl aliquot of this solution (ca. 23.3 μg of IBS). Standard HCl-phenol hydrolysis during 1 hr at 150°C was used to break the amide bonds of the proteins. 5 nmoles each of norvaline and sarcosine were added as internal standards just prior to hydrolysis. The amino acids were separated and quantified on a Hewlett-Packard AminoQuant HPLC analyzer. This technique includes pre-column derivatization using *o*-phthalaldehyde (Godel et al. 1991). Amounts of each amino acid were quantified by comparison of sample peak areas to a calibration plot of known standards. Internal standards were used to control errors due to sample loss and injection variations. Tryptophan could not be detected by this method; asparagine was quantified together with aspartate, and glutamine together with glutamate. The total amount of protein in the sample was estimated based on the concentrations and molecular weights of the amino acid residues.

The estimated total amount of protein varied between the replicates from 45.03% to 62.39% of weight, with the average 57.72%. The data are consistent with the previous results, which indicated that IBS are not entirely composed of proteins (Smith and Littau 1960, Gouranton 1967). At the same time, incomplete removal of contaminants during the sample preparation, incomplete resuspension, or incomplete hydrolysis all could have reduced these values, which therefore should be considered as minimum estimates. The amino acid composition data are summarized in Table 7.1. Analyses of additional samples of IBS of *P. irroratus* using a different instrumentation and a method which allowed to quantify only 15 amino acids produced highly similar results (not shown). IBS are conspicuously glycine- and tyrosine-rich. The content of each of these residues was three times their overall frequency in the Swiss-Prot protein database (Boeckmann et al. 2003). Because the analyzed samples contained an unknown number of different proteins, the compositional data could not be used for protein identification. Nevertheless, it is noteworthy that a high content of glycine is typical of animal fibrous structural proteins, such as keratins, collagens, silk fibroin, elastin, and resilin, found in the structures that combine mechanical strength and elasticity (e.g., Zhou et al. 2001, Tatham and Shewry 2002). Tyrosine plays a role in hardening of some of these polymers by

Table 7.1 Amino acid composition (mol %) of IBS of *Paraphlepsius irroratus*: a summary of three replicates. The abbreviations are as follows: Ala, alanine; Arg, arginine; Asx (asparagine and aspartate); Glx (glutamate and glutamine); Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine

	Ala	Arg	Asx	Cys	Gly	Glx	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Max.	5.32	1.47	7.43	4.5	20.07	8.57	0*	5.31	10.03	0.91	0.45	5.37	9.71	4.42	2.68	na**	10.89	5.98
Min.	5.2	1.3	7.2	4.18	19.19	8.38	0	5.09	9.69	0.68	0.24	5.17	8.15	4.05	2.49	na	10.45	5.77
Mean	5.24	1.41	7.31	4.36	19.6	8.49	0	5.17	9.81	0.82	0.36	5.24	8.91	4.23	2.58	na	10.63	5.85

*below detection limit

**not assessed

cross-linking molecules of their soluble precursors (e.g., Elvin et al. 2005 and references therein). A similar mechanism may be involved in the formation of the IBS walls.

The amino acid composition of IBS differs strongly from that of the phloem (Wilkinson and Douglas 2002) or the xylem (Andersen et al. 1989) plant saps, which are the food source for the majority of leafhoppers (*P. irroratus*, for example, is a phloem feeder). The plant saps display high concentrations of asparagine, aspartate, glutamine, or glutamate and contain relatively small amounts of tyrosine and glycine. However, insects are capable of synthesizing both tyrosine and glycine (Wilkinson and Douglas 2002). The obtained results rule out the possibility, suggested by early authors (Smith and Littau 1960, Wigglesworth 1972), that IBS are merely nitrogenous excreta.

7.4.2 Hydrophobicity of the IBS Coatings

IBS coatings are strongly hydrophobic. Simple experiments demonstrate that a droplet of water does wet the leafhopper integument free of IBS (Fig. 7.5A), but does not wet the integument densely coated with IBS (Fig. 7.5B and C); in the last case the observed contact angles (CA) are close to 180° (Fig. 7.5B). The IBS coating is similarly repellent to aqueous solutions of sugars.

The surfaces displaying CA>150° are referred to as superhydrophobic (e.g., Feng et al. 2002). The value of CA is the product of the chemistry of the surface and its texture. Surfaces are generally referred to as wettable if CA<90° and unwettable if CA>90°. A droplet of water placed between two solid surfaces, such, for example, as insect wings, will pull them together if the surfaces are wettable and push them apart (Fig. 7.5C) if they are unwettable. Adding roughness will increase wettability if the flat surface is wettable and further decrease it if the surface is unwettable. In fact, CA observed on any smooth surface cannot exceed ca. 120°. A further increase of hydrophobicity is only possible by increasing its roughness (reviewed in Quéré 2005). This principle has been extensively studied in plant and insect surfaces

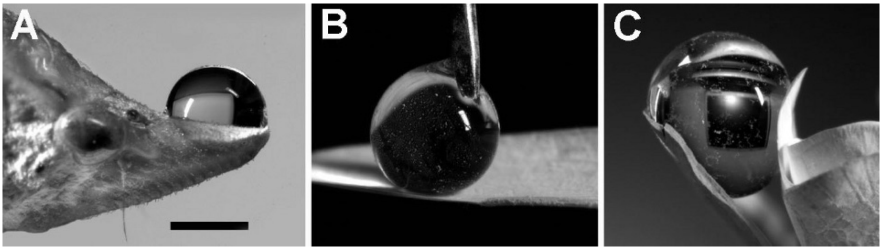

Fig. 7.5 Wetting of the integument of *Diestostemma* sp. with distilled water. This species displays a copious IBS coating on the entire body except the head and the *upper surface* of the pronotum (see Fig. 7.3D). **A**. A droplet resting on the IBS-free surface of the head. Scale bar: 1 mm. **B**. A droplet (attached to a syringe needle) touching the IBS-coated *lower surface* of the forewing. **C**. A droplet placed between the tips of a forewing and a hind wing

(Cassie and Baxter 1945, Fogg 1948, Holdgate 1955, Holloway 1970, Juniper 1991, Wagner et al. 1996, Barthlott and Neinhuis 1997) and, more recently, applied to the design of artificial materials (Shibuichi et al. 1996, Fresnais et al. 2004, Fürstner et al. 2005, Ming et al. 2005). Large values of CA observed on natural surfaces (up to 160–170°) are achieved by development of a microsculpture at the micron and submicron range (Holdgate 1955, Holloway 1970, Juniper 1991, Wagner et al. 1996, 2003). Roughness can be developed to such an extent that the water-covered surface holds air; such "composite" surfaces display extremely large CA. Studies of self-cleaning in superhydrophobic *Lotus* leaves have emphasized the role of the dual-size fractal topography, combining a coarse-scale roughness on the order of 10–20 μm scale with a finer sculpture in the range of 100 nm–1 μm (Barthlott and Neinhuis 1997, Feng et al. 2002). Based on this principle, a superhydrophobic material has been recently created which has the surface coated with raspberry-like particles, each consisting of a 0.7 μm sphere covered with 70 nm spheres (Ming et al. 2005), i.e., precisely in the size range of IBS and their surface compartments.

It is clear now that, even if the material comprising the surface of IBS is only moderately hydrophobic, the IBS layers can be highly water-repellent because they form a complex fractal surface structure in the submicron-to-nanometer range. When covered with water, a layer of IBS may be able to trap the air between the particles, in their wall compartments, and in their central cavities. Indeed, a plastron of air is visible on submerged leafhoppers. Hydrophobic particles can adsorb at the surface of liquid droplets, transforming such droplets into unwettable liquid "marbles" (Aussillous and Quéré 2001), the principle used by the gall-inhabiting aphids to render their honeydew harmless (Pike et al. 2002). When a droplet of water comes into contact with the IBS coating, some loose particles get adsorbed to its surface, but not enough to form a liquid marble. The forces that keep IBS attached to the cuticle and each other have not been studied.

7.5 Adaptive Significance of the IBS Coatings

Because no direct experiments have been carried out yet to elucidate the adaptive value of the IBS coatings, the available hypotheses are largely speculative. It should also be kept in mind that, like most surfaces that form an interface between an organism and the environment, the IBS coatings may have evolved multiple functions. Disentangling their primary and accessory roles in the biology of leafhoppers may, therefore, be a challenging task.

Physiologists who focused their attention on the synthesis of IBS in the Malpighian tubules ignored the fact that IBS form coatings on the leafhopper integument and assumed them to be bizarre-shaped nitrogenous waste products (Smith and Littau 1960, Wigglesworth 1972). However, as mentioned before, the BS-producing cells lack the ultrastructural features typical of the excretory epithelia. Combined with the above data on the amino acid composition of IBS, it makes the excretory function highly unlikely. It is worth pointing out in this connection that similar

IBS are produced by the leafhopper species feeding on phloem (the majority of taxa), xylem (Cicadellinae), and the mesophyll cell contents (Typhlocybinae). Alternatively, Day (1993) has suggested that IBS may carry a pheromone that slowly evaporates through the openings in the wall compartments (Fig. 7.1A). Although Humphrey and Dworakowska (2002) reported that males of some typhlocybine species display significantly more copious accumulations of IBS on their faces than females, it is highly unlikely that IBS contain a sex pheromone. In most leafhoppers IBS are produced and applied to the body, usually in similar quantities, by immatures as well as adults of both sexes. In general, there is no evidence that Cicadellidae produce any kind of pheromones (for discussion about sex pheromones in other Hemiptera see Aldrich (1996)).

The majority of authors suggested that IBS benefit leafhoppers in the form of integumental coatings, which may protect leafhoppers from getting trapped into their sticky liquid excretion or water, from other environmental hazards (desiccation, temperature fluctuations, or solar radiation), pathogens, predators, or parasites (Vidano and Arzone 1984, Arzone 1986, Navone 1987, Günthart 1977, Rakitov 1995, 2002a, Humphrey and Dworakowska 2002). It has also been pointed out in these studies that the IBS coatings are remarkably similar in their appearance to the coatings of particulate wax, produced by other insects, which may indicate similarity in their functions. These hypotheses and the functional analogy between IBS and waxes will be discussed below. A more extensive review of these topics can be found in Rakitov (2002a).

7.5.1 Repelling Water, Honeydew, and other Sticky Substances

Because most leafhopper species live far from water, the adaptive significance of the water-repellent properties of the IBS coatings may not be immediately obvious. Yet, as with all small terrestrial arthropods, contact with rain water or dew may result in the organism becoming trapped by the surface tension. Water can block the spiracles, stick movable body parts together, and stimulate germination of pathogenic fungal spores (see below). While wetting of insect wings with water droplets has been studied in several species (Cassie and Baxter 1945, Holdgate 1955, Wagner et al. 1996), the mechanisms preventing dew condensation on the insect cuticle are poorly known. Hypothetically, the most dangerous for leafhoppers may be dew condensation on the surface of their delicate flying wings, broadly overlapping at rest under the pair of protective forewings. Sticking of the wings to each other or to the abdomen would almost certainly prove fatal.

Perhaps even more dangerous for leafhoppers than water could be their own liquid excretion. The majority of leafhoppers feed on phloem and excrete sugar-containing “honeydew”, which is sticky and provides a substrate for the growth of pathogenic fungi (Pike et al. 2002 and references therein). The excrement produced by mesophyll-sucking Typhlocybinae is similar (personal observation), and that of Cicadellinae and several additional, smaller xylem-feeding lineages is more watery, but also far more copious (Brodbeck et al. 1993).

Various sap-feeding Hemiptera display diverse mechanisms of protection against honeydew: incorporating the excretion into the scale covers in armored scale insects (Foldi 1982), coating the honeydew droplets with a layer of wax in immature psyllids (Weber 1930) or with wax powder in gall-inhabiting aphids (Pike et al. 2002), removing droplets from the anus with the legs in some aphids (Kunkel 1972), and shooting excreta from the anus in aphids, psyllids, whiteflies, planthoppers, leafhoppers, and related groups (Weber 1930, Kunkel 1972, Strümpel 1983). Additionally, the integument of many aphids, whiteflies, scale insects, and planthoppers is copiously coated with particulate wax, which renders them invulnerable to the honeydew (e.g., Pike et al. 2002). All leafhoppers forcibly shoot droplets of their excrement away. Yet, in denser populations, they are at risk of being contaminated with the neighbor's excrement directly or through the contaminated plant surface.

Rakitov (2002a) has summarized observations on the interspecific variation in the development of the IBS coatings which appear to corroborate these hypothetical functions. Leafhopper species occurring in moist habitats often display better developed coatings. In particular, among species occurring in Europe, the most dense and conspicuous IBS coatings are found in the only species living on leaves of floating plants, *Erotettix cyane* (Boheman) (Rakitov 1995). The galls of the only known leafhopper species producing true galls, *Scenergates viridis* (Vilbaste), are filled with copious IBS, originally described as the "white waxy substance" (Mitjaev 1968). Wetting and sticking are especially hazardous for small insects, because such insects may not be able to overcome the surface tension of the liquids. Remarkably, the reserves of IBS in the form of spots on the forewings (Fig. 7.3A) are most commonly found in the subfamilies Typhlocybinae (Vidano and Arzone 1984) and Xestocephalinae (Rakitov 2000a), which comprise small, delicate species.

The layers of IBS may provide protection against water and sticky substances not only by reducing the wettability of the cuticle, but also due to their particulate nature. Because of the loose attachment of the particles to each other and the integument, a body part coated with IBS and brought in contact with water or a sticky substance will more likely to lose some IBS rather than become trapped. By the same principle, the IBS coatings may also help leafhoppers escape from spider webs, similarly to Lepidoptera that sometimes escape leaving but a few scales on the adhesive threads (Nentwig 1982). Of equal importance may be protection from sticky glandular trichomes on the surface of plants (Tingey 1985) and from sticky spores of certain entomopathogenic fungi (see below).

7.5.2 Preventing Desiccation

It has been hypothesized that, like for small terrestrial arthropods in general, reduction of water loss is important for leafhoppers, and consequently it may be the major function of their IBS coatings (Arzone 1986, Humphrey and Dworakowska 2002). Theoretically, the IBS coatings may reduce transpiration through the cuticle either directly, as a lipid-containing meshwork on the surface, or by forming around the integument a boundary layer of unstirred air, as has been suggested for particulate waxes of certain insects (Hadley 1979, 1994). Sealing of sutures and intersegmental folds with IBS must also reduce water loss. Nevertheless, the ecology of leafhoppers does not suggest that desiccation is a crucial factor for most species. Leafhoppers, as a group, occur in a variety of habitats, from extremely dry to moist and, like all plant-sucking insects, receive excessive amounts of water with their food.

7.5.3 Protection Against Fungal Pathogens

Leafhoppers can be infected by many species of pathogenic fungi (e.g., Müller 1956, Mitjaev 1963, McGuire 1985, Soper 1985, Rombach et al. 1987, Galaini-Wraight et al. 1991, Wraight et al. 1990, 2003). Initial events of fungal infection in insects include non-specific adsorption of the spore to the insect cuticle, host recognition, consolidation of attachment, germination and growth on the surface and, finally, penetration into the body. Entomopathogenic spores range from dry hydrophobic to sticky hydrophilic; they can disperse by air or by rain splashes, or they will adhere to a passing insect (Boucias and Pendland 1991). IBS may directly protect leafhoppers if they contain fungistatic or fungicide substances, such as have been identified in the cuticle of some insects (Smith and Grula 1982, Sosa-Gomez et al. 1997), or as a mechanical barrier against the spore attachment and subsequent penetration. As a result of grooming, IBS often caulk sutures and intersegmental folds, which serve as portal of penetration for some of the pathogenic fungi (McGuire 1985, Wraight et al. 1990). Because the spores of the fungus species known to infect leafhoppers are 3–100 times larger than ca. 0.4 μm IBS found in most leafhoppers (Soper 1985) only a dense layer of IBS is likely to keep the spores far enough from the cuticle to prevent their germination. The spores trapped in such coatings may be subsequently brushed away during grooming. *Zoophthora* and some other entomopathogenic fungi form specialized passively distributed spores, capilliconidia, which secrete droplets of viscid fluid at their tips and adhere to a passing insect (Glare et al. 1985, Boucias and Pendland 1991). In this case, as well as in the case of the pathogenic spores distributed with rain splashes, the IBS coatings may provide efficient protection. In order to germinate and penetrate the host, the spores generally require moist conditions (e.g., Wraight et al. 1990). The IBS coatings may therefore protect leafhoppers from fungal pathogens indirectly by keeping the integument dry.

7.5.4 Other Hypothetical Functions

It has also been suggested that the layers of IBS may reflect excessive ultraviolet radiation or serve as a thermal insulation (Navone 1987). The IBS coatings indeed reflect UV light (Rakitov unpublished), but in most cases appear to be too thin to buffer fluctuations of temperature. Several desert leafhopper species display copious IBS coatings on the exposed surfaces (e.g., *Achrus ahngeri* (Melichar) and

Cuerna yuccae Oman and Beamer). Neither excessive sunlight nor heat, however, appear to be principal hazards for the majority of leafhoppers. The IBS coatings of most leafhoppers are too thin to deter predators or parasites as a simple mechanical barrier. However, the possibility that these particles disrupt attachment of the enemies to the leafhopper cuticle (see Eigenbrode et al., this volume, and references therein) merits further study. Several hypotheses have been advanced to explain how minute surface structures may prevent attachment of insect tarsal pads (Gorb and Gorb 2002).

7.5.5 Comparison with Wax

Although particulate extracuticular waxes can be found in several insect orders, they are especially common and diverse in Hemiptera. Here copious amounts of wax are produced by some planthoppers (O'Brien and Wilson 1985, Mason et al. 1989, Rakitov 2002a, Lucchi and Mazzoni 2004), scale insects (Foldi 1991), aphids (Pope 1983, Smith 1999), psyllids (Hodkinson 1974), and whiteflies (Navone 1987, Byrne and Hadley 1988, Nelson et al. 1999). The hemipteran waxes are mixtures, which may contain long-chained aldehydes, alcohols, esters of alcohols and fatty acids, free fatty acids, or hydrocarbons, depending on the species and location on the body (Mason et al. 1989, Foldi 1991, Buckner et al. 1999, Nelson et al. 1999). The wax particles or filaments display a dazzling variation of sizes and shapes and often are intricately structured in the micron-to-submicron size range (Pope 1983, Navone 1987, Byrne and Hadley 1988, Smith 1999, Rakitov 2002a, Nelson et al. 2003), which at least in some cases accounts for the superhydrophobic properties of their masses (Pike et al. 2002). Location and external appearance of the wax are often similar to those of the IBS coatings. Moreover, it has been shown by Navone (1987) that dustywings (Neuroptera: Coniopterygidae), whiteflies (Hemiptera: Aleyrodidae), and leafhoppers display remarkably similar behavioral and morphological specializations involved in making integumental coatings of intricately structured wax particles in the first two groups and of IBS in the third. Therefore, particulate waxes and IBS may have similar functions despite profound differences in their morphology, composition, and origin (Arzone 1986, Navone (1987), Rakitov 1995, 2002a).

In fact, all of the hypothetical functions proposed for IBS have also been proposed for the hemipteran waxes: excretion (Pollister as cited in Pope 1983, p. 495), carrying pheromones (Byrne and Hadley 1988), and protection against parasites, predators, fungal pathogens, excessive heat, harmful radiation, desiccation, and water, but most importantly from honeydew (Hodkinson 1974, Pope 1983, 1985, Navone 1987, Byrne and Hadley 1988, Foldi 1991, Smith 1999, Pike et al. 2002). In most cases these hypotheses have been derived from comparisons between life histories of the species producing copious wax and those that produce little or no wax. More recently, however, experimental studies have been conducted on the anti-sticking function of the wax protecting gall-inhabiting aphids from their honeydew (Pike et al. 2002 and references therein) and the role of waxy blooms as a camouflage in some free-living aphids preyed on by spiders (Moss et al. 2006). Additionally, the anti-desiccation function has been studied in some desert tenebrionid beetles (Hadley 1979, McClain et al. 1985, 1986), and the correlation between aridity of the habitat and presence of wax blooms in the genus *Onymacris* has been tested statistically in a phylogenetic framework (Ward and Seely 1996).

Among hemipteran lineages closely related to leafhoppers, conspicuous amounts of particulate body wax are produced in certain treehoppers (Membracidae) and cicadas (Cicadoidea). No species of Cicadellidae is known to secrete copious wax, which is remarkable given the extreme diversity of the family. This pattern is consistent with the hypothesis that IBS and waxes have analogous functions. At the same time, small amounts of particulate wax have been found in some leafhopper species (Fig. 7.4I, see also Rakitov 1995), indicating that wax and IBS can coexist.

7.6 Egg Brochosomes (EBS)

The use of BS as a coating to egg masses is restricted to a group of 18 related New World genera, most of which are currently placed into the tribe Proconiini (Swain 1936, Hix 2001, Rakitov 2004, Rakitov and Godoy 2005, Azevedo-Filho and Carvalho 2005). Together these genera contain approximately 200 species or less than 1% of the global diversity of leafhoppers. Prior to oviposition, females from these genera place droplets of the Malpighian tubule secretion onto their forewings, where the liquid dries into a pair of white, usually bulgy pellets (Fig. 7.6A). An

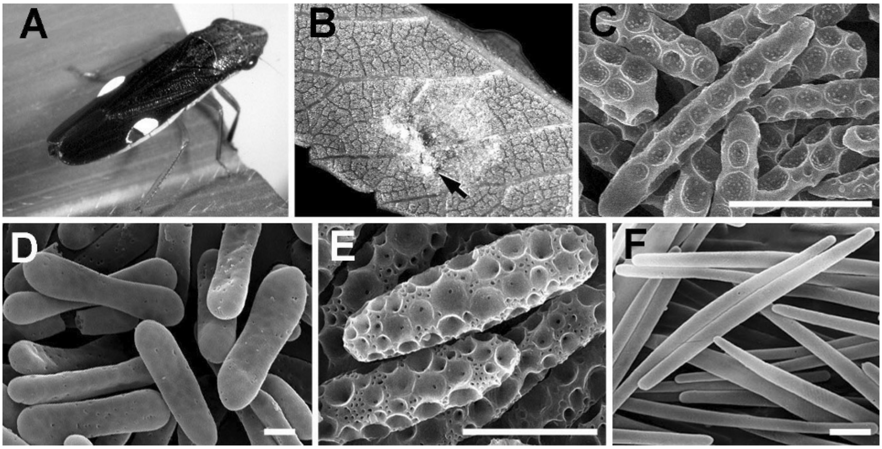

Fig. 7.6 EBS and their use. **A**. Female *Homalodisca insolita* (Walker) with a pair of EBS masses on the forewings. **B**. An egg nest of *H. vitripennis* (Germar) on the lower suface of a leaf; the arrow points at the more copious coating directly above the oviposition slit. **C**. EBS of *H. insolita*. **D**. Same, *H. noressa* Young. **E**. Same, *Cuerna arida* Oman and Beamer. **F**. Same, *Acrogonia* sp. Scale bars: C–F, 2.0 μm

egg-laying female cuts a slit in the epidermis of a plant leaf, injects the eggs, and then uses its hind legs to scrape BS off the wings onto the slit and surrounding area (Fig. 7.6B). In several species demonstrating this behavior the BS used in oviposition are morphologically identical to IBS (e.g., in *Homalodisca liturata* (Ball) and *Tapajosa* spp., Rakitov 2004), but more typically the Malpighian tubules of the gravid females switch over from production of regular IBS to production of larger, usually elongated particles (Rakitov 2000b). Such BS can reach 20.0 μm in length; their size and structure vary dramatically among species (Fig. 7.6C–F, see also Rakitov 2004). It is convenient to refer to both the specialized and regular BS applied onto egg nests as one functional type, EBS. It has been found that EBS can impede parasitization of the eggs by chalcidoid wasps (Hymenoptera, Mymaridae) by sticking to the bodies of the female wasps and triggering frequent bouts of grooming (Velema et al. 2005). Rakitov (2004) has argued that the elongate shape of EBS facilitates storing large masses of this material on the wings and its transfer onto the egg nests by increasing cohesion between the particles. At the same time, more elongate and larger particles may inflict greater irritation to the parasitoid wasps. EBS are hydrophobic (Rakitov unpublished). Therefore, by sealing the slits, EBS may prevent flooding of the eggs by plant fluids and ensure penetration of air to the developing embryos. EBS may also play a role in decreasing water loss through the slits.

Application of waxy blooms to eggs, laid exposed or inside live plant tissues, is known in various Hemiptera (Weber 1930, Metcalfe 1968, O'Brien and Wilson 1985, Navone 1987, Nelson et al. 1999, Rakitov 2002a) and Coniopterygidae (Navone 1987), but its functions have not been studied in detail.

7.7 Origin and Evolution of BS

It should be clear from the preceding discussion that production of IBS in Cicadellidae involves a complex of unique physiological, structural, and behavioral traits. Presence of IBS on the cuticle has been recognized as one of the few autapomorphies of this family (Deitz and Dietrich 1993). A major diagnostic trait of leafhoppers, the elaborate setal armament of the legs, is intimately associated with creation and maintenance of the IBS coatings (Rakitov 1998). It stands to reason to assume that BS have played a role and perhaps even facilitated the spectacular diversification of this family. Yet, the assumption that the IBS coatings of Cicadellidae are functionally analogous to particulate wax coatings of other insects (see above) creates an interesting paradox. The epicuticle of insects generally includes a thin layer of wax which forms the exposed surface or lies under a still thinner cement layer, depending on the species (Chapman 1998). This wax is produced by specialized epidermal cells and is released across the deeper cuticular layers through a system of canals. From the evolutionary standpoint, secretion of the extracuticular wax in form of particles or filaments is a mere elaboration of this process, which explains why it is found in a variety of distantly related insect lineages. As mentioned before, the integument of leafhoppers also displays this obviously ancient capability. Then why should leafhoppers have evolved IBS? One possible explanation is that the hypothetical precursors of IBS might have had a different, currently unknown biological function, and only later took over the more common functions of particulate extracuticular coatings (Rakitov 2002b).

Among modern Cicadellidae, IBS have been found in all of the major subfamilies (Rakitov 1995, 1998), suggesting that no leafhopper lineages currently exist that had branched off before IBS evolved. Cicadellid fossils from the Lower Cretaceous display well differentiated setal armament of the legs (Hamilton 1990), suggesting that these insects already actively coated their integument with particles, possibly IBS. A glance into still earlier evolution of BS production can be obtained by comparing Cicadellidae with related extant lineages of Hemiptera. In fact, glandular specializations of the Malpighian tubules are characteristic of all the three major lineages of the infraorder Cicadomorpha: Cercopoidea (spittlebugs), Cicadoidea (cicadas), and Membracoidea (leafhoppers and treehoppers) (Rakitov 2002b). In both spittlebugs and cicadas, these organs synthesize secretory products during the nymphal stage only, and such products are released with the main flow of excreta. In spittlebugs they make a part of the characteristic “spittle” surrounding developing immatures (e.g., Marshall 1966, 1973), and in cicadas they make a part of the “anal liquid” that is used to strengthen the walls of the nymphal burrows and clean the body (Boulard 1965, Kudryasheva 1979). Because application of the Malpighian tubule secretory products to the integument is, therefore, shared by members of all the extant lineages of Cicadomorpha, Rakitov (2002b) suggested that this trait is plesiomorphic in these taxa. He conjectured that the nymphs of early cicadomorphans were subterranean and xylem-feeding, like those of extant cicadas and many spittlebugs, and that their Malpighian tubules were modified to synthesize products that possibly prevented the growth of fungi in the soil moistened with the nymphal excretion, neutralized toxic waste products contained in the nymphal excretion, or optimized the pH of the substrate. In the lineage leading to the extant Membracoidea, the nymphs switched to the open and agile life style, but apparently retained the habit of applying the Malpighian tubule products to the integument, which could have eventually led to evolution of IBS instead of elaborate protective extracuticular waxy coatings. The additional function of BS as a coating to egg nests (EBS) has evolved within a single lineage of leafhoppers via modification of the basic complex of traits involved in the synthesis and application of IBS (Rakitov 2004). According to Dietrich et al. (2001), treehoppers (Membracidae, Aetalionidae, and Melizoderidae) have derived from within Cicadellidae and lost the production of IBS secondarily; the function of anointing behaviors observed in this group (Rakitov 1996) is enigmatic. The traits in which treehoppers differ from leafhoppers and that may correlate with the absence of the IBS coatings include the heavier sclerotization of the body, more sedentary life style, production of copious wax on certain body parts (in some taxa), and generally more pronounced microtextures of the cuticle (e.g., Dietrich 1989).

7.8 Conclusions and Perspectives

The coatings formed by IBS on the integument of Cicadellidae are strongly hydrophobic, apparently in large part due to the extremely complex fractal geometry of their surface at the micron-to-nanometer scale. These coatings may serve multiple biological functions analogous to those of particulate wax coatings of other insects. At the same time, among several such functions hypothesized, only protection of the integument from wetting by water and leafhoppers' own liquid excreta can explain the nearly universal occurrence of the IBS coatings in this diverse insect family. The method of isolation of IBS for chemical analysis with organic solvents, described in this chapter, opens ways for more detailed characterization of the protein component of IBS, which will shed additional light on the biological function and evolutionary origins of these structures. However, other approaches need to be found to characterize the lipids contained in the IBS.

Experimental studies are also needed to elucidate the behavior of the IBS layers during water condensation and during contact with water and aqueous solutions similar in their composition to honeydew. Obtaining IBS-free integuments for comparison in such experiments may be possible by preventing transfer of the Malpighian tubule secretion onto the cuticle after molts (Rakitov, unpublished). A comprehensive study of grooming behavior can also contribute important insights into the function of the IBS coatings. Better understanding of the properties of the IBS coatings can inspire the design of artificial superhydrophobic materials. Conversely, the current research on such artificial surfaces can help in understanding biologically important properties of the IBS coatings in leafhoppers and other poorly studied extracuticular features of insect integuments.

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