

What are brochosomes for? An enigma of leafhoppers (Hemiptera, Cicadellidae)

R. A. RAKITOV

Abstract

Production of brochosomes is an enigmatic trait unique to leafhoppers (Cicadellidae). These curiously structured ultra-microscopic protein-lipid particles are produced in the specialized cells of the Malpighian tubules, but it is unlikely that they are an excretory product. Leafhoppers actively apply brochosomes to their integument and, sometimes, to their egg nests. The small size and intricate surface structure of brochosomes apparently render layers of these particles unwettable with water and sticky honeydew. Another possible function of such coatings is direct or indirect protection against the attachment and germination of fungal spores. It is unlikely that any of the other proposed roles, that include the protection from desiccation, UV light, temperature fluctuations, and from predators and parasites, is the major function of this secretion in the extant Cicadellidae. None of the hypothetical roles of brochosomes has yet been investigated experimentally. All such roles, however, suggest that brochosomes

are functionally analogous to the waxy particulate coatings of epidermal origin on the integument and eggs of various insects. The synthesis of secretory products by the Malpighian tubules and the habit of applying these products on the integument may have evolved in the ancestral Cicadomorpha as an adaptation to a subterranean habitat of the immatures. These traits may have been preadaptations to using such products, rather than epidermal waxes, as a protective coating when the immatures of early Membracoidea switched to free-living. The inadequate knowledge of the properties of brochosomes and complete lack of experimental studies render the current interpretations highly speculative. To fully elucidate the function of brochosomes, future studies should employ diverse experimental and comparative approaches.

Key words: Hemiptera, Cicadellidae, brochosomes, integument, eggs, Malpighian tubules, wax, secretion, structure, behavior, function, evolution.

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1. Introduction

Leafhoppers (Cicadellidae) can be characterized as organisms coated with brochosomes. The known exceptions are immatures from certain subfamilies, that do not produce brochosomes until the last instar, and a few aberrant species. Production of this unique biomaterial sets leafhoppers apart from the rest of Auchenorrhyncha and insects in general. Leafhoppers actively apply brochosomes on and spread them over the body using modified legs. Apparently, brochosomes play a certain role in the life of leafhoppers. What might this role be? Answering this question is integral to understanding the ecology and physiology of this largest family of the extant exopterygote insects as well as the causes of its spectacular radiation. Yet, fifty years after the discovery of the "ultramicroscopic bodies" (TULLOCH et al. 1952) their properties remain poorly known, and their function is essentially enigmatic. This contribution briefly reviews what is known about brochosomes from the perspective of their function and discusses the hypotheses aiming to explain it.



Fig. 1. A female of *Draeculacephala* sp. and its Malpighian tubules, shown at the same scale. The blind posterior ends of the two left tubules are attached to the rectum. The tubule products are released through the anterior ends that open into alimentary canal between the midgut and the hindgut (not shown). The relatively large size of the brochosome-secreting segments (arrow) may be viewed as an indirect indication of the important role played by brochosomes. Bar = 1mm

2. Brochosomes

2.1 Brochosomes on Integument

Brochosomes are protein-lipid particles synthesized in the glandular segments of the Malpighian tubules of leafhoppers (Fig. 1). Size and structure of brochosomes vary among species, but in the majority of examined leafhoppers they are hollow spheres, 0.2-0.6 μm in diameter, with a honeycomblike surface (Fig. 2). The bottoms of the hexagonal and pentagonal surface compartments usually have openings leading into the central cavity. Brochosomes originate in Golgi complexes and acquire their final shape before leaving secretory cells (DAY & BRIGGS 1958; SMITH & LITTAU 1960; GOURANTON & MAILLET 1967; CHEUNG & PURCELL 1991; RAKITOV 1999a, 2000a).

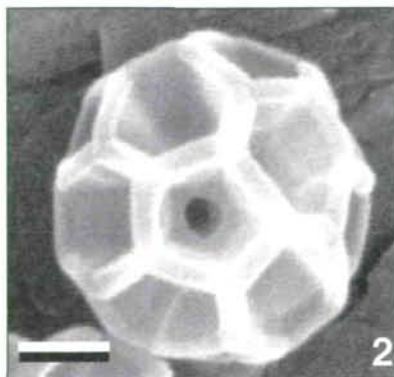
After molts, leafhoppers release a colloidal suspension of brochosomes through the hindgut and apply it on the new integument. Details of this behavior, referred to as anointing, vary between species and also between

immatures and adults of the same species (STOREY & NICHOLS 1937; NAVONE 1987; RAKITOV 1996, 2000b). 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 a few more droplets, transfers them onto the forewings, and spreads them over the dorsal side of the forewings, pronotum, and head (Figs 3, 4). The fluid dries leaving a sediment of brochosomes. Anointing is followed by vigorous grooming: the leafhopper rapidly rubs and brushes its body and appendages. During this process brochosomes are redistributed more evenly across the integument. In particular, in adults, brochosomes are scraped from the dorsal forewing surface onto the hindwings and the dorsal abdomen. Rows and groups of strong setae on the legs of leafhoppers (Fig. 5) serve as minute rakes or brushes for manipulating the brochosome powder (VIDANO & ARZONE 1984; NAVONE 1987; RAKITOV 1998). Anointing is



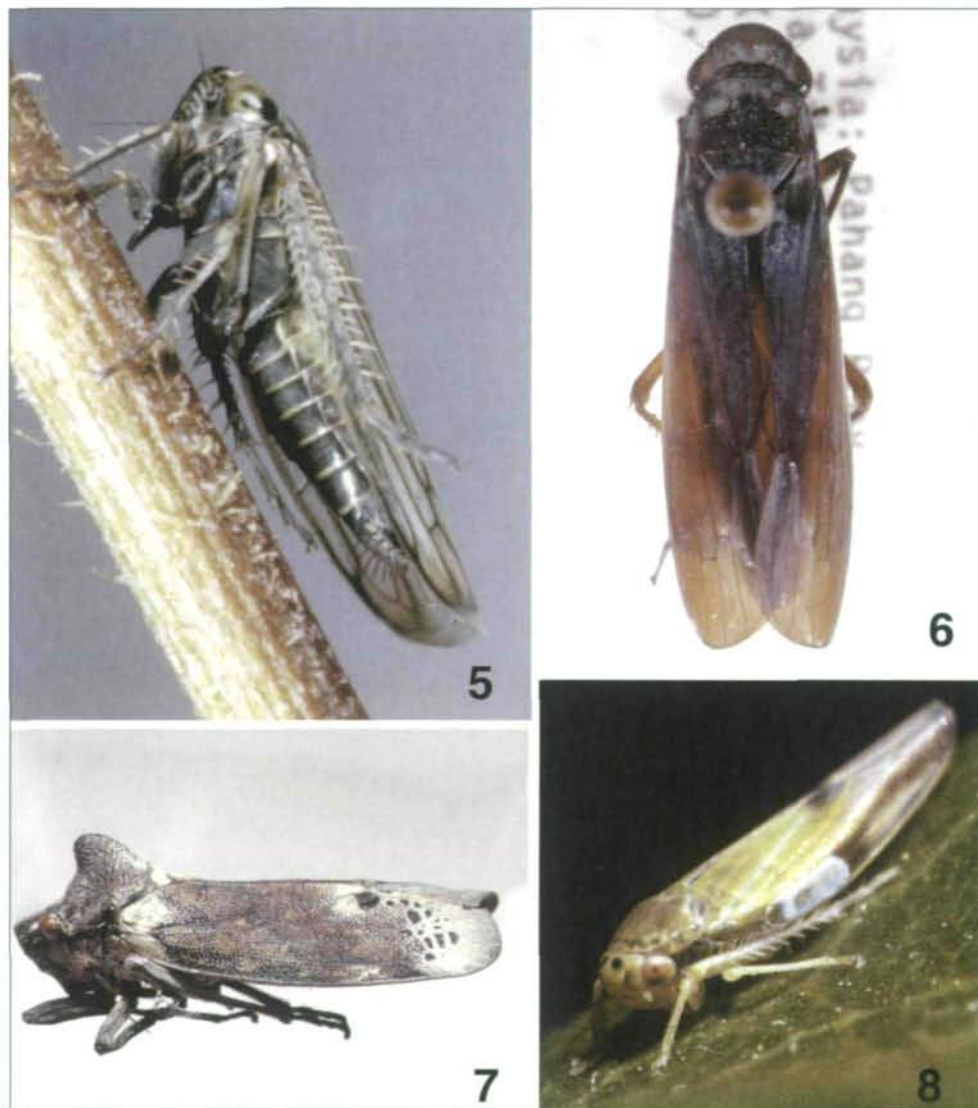
Figs 3-4. Successive moments of anointing in *Oncopsis flavicollis* (L.). **Fig. 3.** The brochosome-containing secretion is being released from the anus and picked up with the hind legs. The fluid appears opaque because of the presence of brochosomes. **Fig. 4.** The hind legs are spreading this fluid over the dorsal integument.

Fig. 2. A brochosome of *Paraphlepsius irroratus* (SAY). Particles of this type are produced by the majority of the examined leafhopper species. Bar = 100nm.



usually observed within 1-3 hours after the molt before the leafhopper starts feeding. It may be repeated several times; adults apparently retain the ability to secrete brochosomes throughout their lifetime (RAKITOV 1996). In the leafhoppers that have the integument already coated with brochosomes, bouts of grooming are observed as a stand-alone behavior.

can be done under the stereomicroscope by touching the integument with a preheated entomological pin; melting of the particulate wax is readily seen, while the layer of brochosomes remains unchanged. To draw a final conclusion, however, the material should be studied under the electron microscope. A few known cases of wax production in leafhoppers will be touched on below. Brochosomes can be



Figs 5-8. External appearance of brochosome coats.

Fig. 5. *Exitianus exitiosus* UHLER. A very thin light deposit of brochosomes can be noticed on only the dark parts of abdomen, thorax, and legs. The bluish tint of the coat is created by scattering of light by the particles close to $0.5\mu\text{m}$ in diameter, known as the TINDALL effect.

Fig. 6. *Sochinsogonia robonea* YOUNG. The brochosome coat is conspicuous on the dorsal surface of the forewings as well as on the other body parts.

Fig. 7. *Proconia* sp. The white brochosome powder is very conspicuous on the brown cuticle of this male specimen.

Fig. 8. *Vilbasteana oculata* (LINDB.) displays reserves of brochosomes in the form of white oval spots in the costal part of each forewing (only left spot is visible in the photo). Often referred to as "wax-areas", such spots are common among Typhlocybinae.

The brochosome coat varies in continuity and thickness among species, individuals, and body parts of the same individual from scattered particles to a dense layer (Figs 5-14). It may be visible in the stereomicroscope as a pale, non-shiny thin deposit on the darker body parts (Fig. 5). Thicker coats, visible by naked eye, occur more rarely (Figs 6, 7) and resemble particulate waxy coats produced by the epidermis of various insects. A quick test

found on all of the body parts, including antennae (Figs 9-14), but the eyes usually remain clean, and the dorsal surface of the forewings, from which brochosomes are transferred to other body parts, often is almost bare (RAKITOV 1995). As a modification of the typical adult anointing described above, some species do not spread the droplets of the brochosome suspension but let them dry on the forewings as a pair of spots (Fig. 8).

These spots, referred to in older literature as “wax areas”, serve as temporary reserves of ready-to-use secretion. From here brochosomes are spread across the entire body surface through grooming (VIDANO & ARZONE 1984; NAVONE 1987; RAKITOV 2000b).

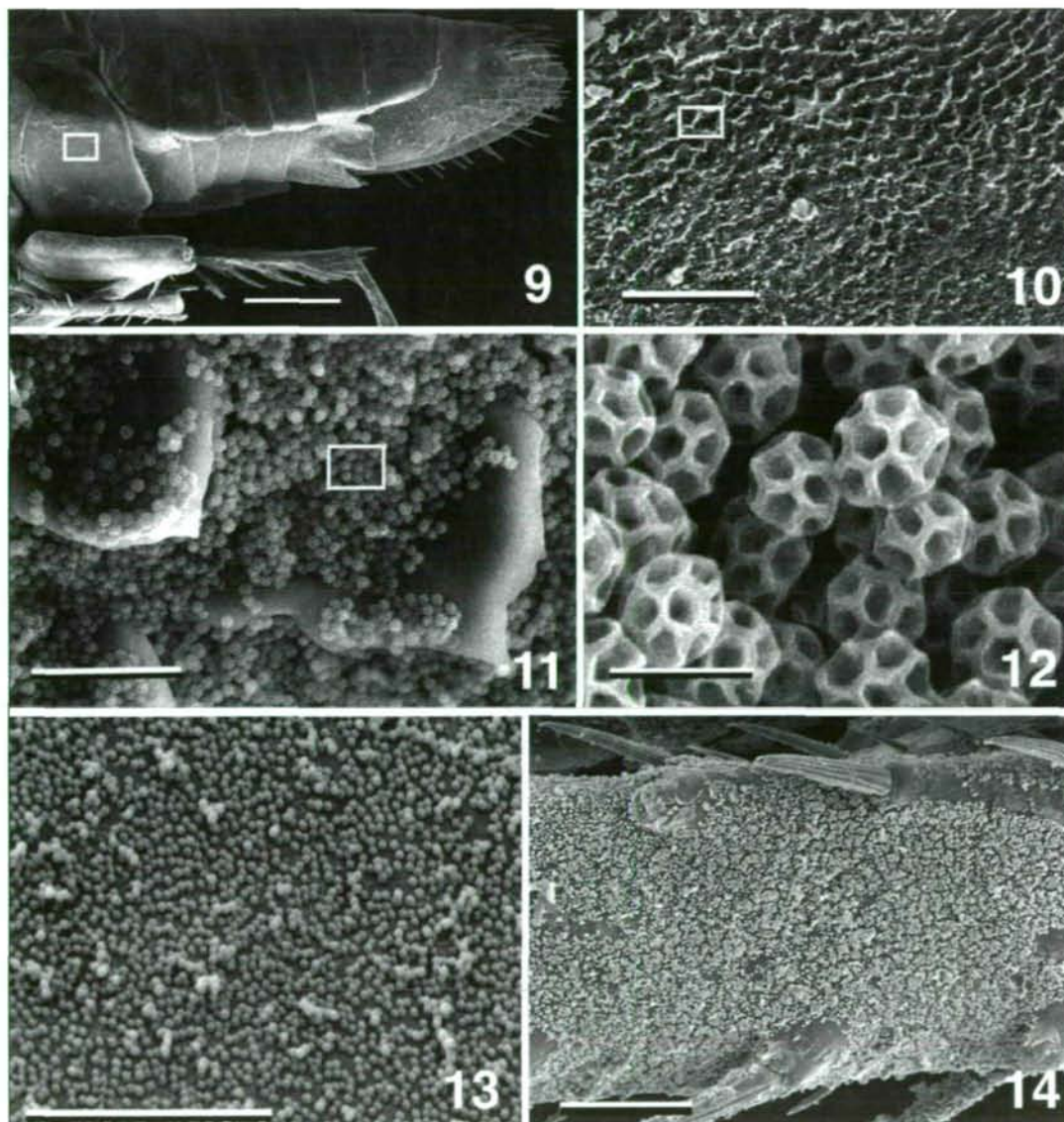
I have examined the integument in adult representatives of the majority of the currently recognized subfamilies of Cicadellidae

rity of the examined subfamilies produce brochosomes. These have not been found, however, in the nymphs of Idiocerinae, Macrospinae, Ulopinae, Agalliinae, and Typhlocybinae. The nymphs of the studied Idiocerinae did not display anointing behaviors (NAVONE 1987; RAKITOV 1996), while those of the other four subfamilies anointed themselves with Malpighian tubule secretions lacking

Figs 9-14. Brochosomes on the integument. Figs 9, 10, 11, 12. *Paraphlepsius irroratus* (SAY), zooming into the hind coxa. White rectangles in Figs 9, 10, and 11 display the field of view at the next magnification. Bars: Fig. 9—500µm; Fig. 10—50µm; Fig. 11—5µm; Fig. 12—0.5µm.

Fig. 13. Brochosome coat on the ventral forewing surface of an adult *Xestoccephalus desertorum* (BERG). Bar = 10µm.

Fig. 14. The outer surface of the hind tibia in a 5th instar nymph of *X. desertorum* is also coated with brochosomes. Bar = 50µm.

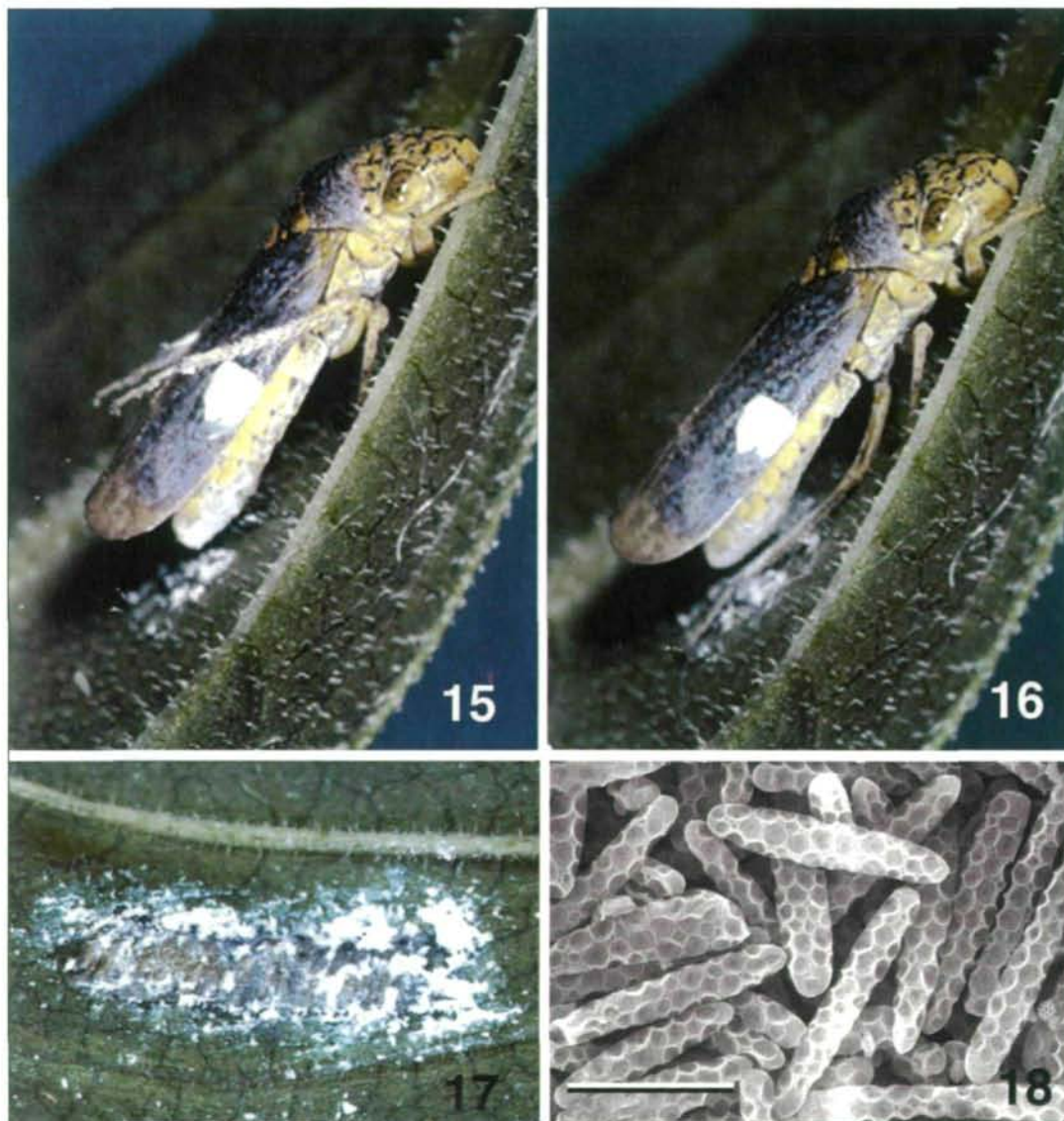


(RAKITOV 1995, 1998). Brochosomes have not been found only in several species of *Iassus*, *Ledra*, *Xerophloea*, and *Cephalelus*. They have been found, however, in other Iassinæ, Ledrinae, and Ulopinae, suggesting that the species lacking the trait lost it secondarily. In *Myerslophia*, recently placed into a separate family (HAMILTON 1999), brochosomes also have not been found. Nymphs from the majo-

brochosomes (RAKITOV 1996, 1999a). Nevertheless, scattered brochosomes can be seen on the micrographs of the integument of the last instar nymphs of *Empoasca fabae* Harris (MCGUIRE 1985), indicating that not all typhlocybina nymphs lack brochosomes. Anointing behaviors similar to those of leafhoppers have been observed in treehoppers

(Membracidae): adult *Heteronotus* (EKKENS 1972, p. 267), immature and adult *Gargara* (RAKITOV 1996), and adult *Platycotis* (RAKITOV unpublished). Treehoppers have in their Malpighian tubules glandular segments similar to those of leafhoppers, but these synthesize secretory products distinct from brochosomes (RAKITOV unpublished). Such products are apparently applied onto the integument

of the tribe Proconiini (Cicadellinae) are known to powder such egg nests with brochosomes (Figs 15-18) (SWAIN 1937; TURNER & POLLARD 1959; RAKITOV 1999b and unpublished; HIX 2001). Immatures, males and virgin females of these species produce typical spherical "brochosomes-for-integument" (see above) and spread them over the body, while the gravid females produce morphologically



Figs 15-18. Use of brochosomes in oviposition in *Oncometopia orbona* (F.). Figs 15, 16. A female scrapes brochosomes from the white pellets present on its forewings onto the oviposition site with synchronous strokes of the both hind tibiae.

Fig. 17. The egg nest on the adaxial side of a *Silphium* sp. leaf several days after oviposition. The eggs with dark embryonic eye spots are partly visible through the plant epidermis. The epidermis is unevenly coated with white brochosome powder.

Fig. 18. Elongate "brochosomes-for-eggs". Such particles comprise the pellets on the forewings of the egg-laying females and the coat on the egg nests. Bar = 5 μ m.

during anointing but form no particulate coatings. No brochosomes have been found on the integument of treehoppers (DIETRICH 1989).

2.2 Brochosomes on Egg Nests

Most leafhoppers insert their eggs into living plant tissues. Species from several gene-

distinct particles, "brochosomes-for-eggs" (Fig. 18) (RAKITOV 2000a; HIX 2001). Shortly before oviposition, a female places droplets of the brochosome suspension onto its forewings, where they dry as a pair of conspicuous white pellets. During oviposition, the female scrapes this material with its hind tibiae onto the oviposition site (Fig. 15, 16), making a pulverulent coat (Fig. 17).

2.3 Chemical Properties

Brochosomes have not yet been isolated and analyzed with modern biochemical methods. Most of the available data on their composition are derived from histological studies. The brochosome-secreting cells display an extensively developed rough endoplasmic reticulum, indicating production of large quantities of protein (SMITH & LITTAU 1960; GOURANTON & MAILLET 1967; RAKITOV 1999a, 2000a). Histochemical tests demonstrated that brochosomes contain both protein and lipid. One study suggested that brochosomes comprise a protein skeleton coated with saturated lipids (SMITH & LITTAU 1960), but another found that they contain proteins and unsaturated phospholipids that are not spatially separated (GOURANTON & MAILLET 1967). GOURANTON (1967) detected an alkaline phosphatase in developing brochosomes in *Cicadella viridis* (L.) but not in two other species. MAYSE (1981) tested masses of "brochosomes-for-eggs" collected from the forewings of *Oncometopia orbona* (F.) for the presence of several common arthropod excretory products and detected allantoin and urea. Brochosomes are remarkably resilient: they are not soluble in organic solvents (GOURANTON & MAILLET 1967); in the dry form they are rigid and durable.

3. Function of Brochosomes

3.1 Problems and Hypotheses

Nothing currently known about the habitats, life history, feeding mode, locomotion, communication, protection, or reproduction of Cicadellidae appears to explain the function of brochosomes. Most of the biological features of the family can be found among the rest of Auchenorrhyncha, for example, in Delphacidae. Do brochosomes have a special function or are they merely excretory products? Or, perhaps, they are excretory products that occasionally serve accessory functions? Is the intricate structure of brochosomes finely tuned to perform certain function? Or, is their shape the artifact of a peculiar crystallization process? Do the brochosomes covering egg nests have the same function as the brochosomes coating the integument? In general, why do leafhoppers produce brochosomes while

other insects do not? And why do not some species or stages of leafhoppers produce brochosomes? A satisfactory explanation of the role of brochosomes must address each of these questions. Different authors interpreted brochosomes as waste products, a protective material, or as reservoirs of hypothetical pheromones. Below, these hypotheses will be considered in more detail.

3.2 Brochosomes and Excretion

Because brochosomes contain protein and are produced in the Malpighian tubules, the primary excretory organs of insects, it has been suggested that they are bizarre nitrogenous waste products (SMITH & LITTAU 1960; WIGGLESWORTH 1972). This interpretation seemed to gain support from histochemical tests that indicated that the Malpighian tubules of leafhoppers contained neither urates, nor any other end products of nitrogen metabolism commonly found in terrestrial insects (SMITH & LITTAU 1960). The qualitative tests of MAYSE (1981), that detected allantoin and urea in the "brochosomes-for-eggs", have not been published in full and do not indicate whether these compounds are major constituents in the material. More recent studies found the regular watery excretion of Proconiini to contain very dilute concentrations of allantoin and uric acid but high concentrations of ammonia, suggesting that the latter is the primary waste product in these insects (BRODBECK et al. 1993). Because these studies did not take into account production of brochosomes, the conclusion that proconiines are ammonotelic should be reassessed when the composition and the amount of brochosomes produced in the Malpighian tubules are known. While the relations between the nutritional physiology of Cicadellidae and synthesis of brochosomes remain obscure, it is clear that there is no direct connection between a particular diet and the production of brochosomes. The leafhopper species feeding on xylem (Cicadellinae sensu lato), mesophyll (most Typhlocybinae), and phloem (the rest of leafhoppers) often produce brochosomes of similar structure. Moreover, immatures of several subfamilies do not produce brochosomes, while sharing the diet with the brochosome-producing adults (RAKITOV 1995, 1996, 1999a).

In other insects, not everything that comes out of the Malpighian tubules is an excretory product. These organs appear to be apt to evolving secondary functions quite unrelated to excretion, and in one case even function as luminescent organs (GREEN 1979). Malpighian tubules modified to perform an accessory synthetic function are known in various insect groups. For example, specialized segments of the tubules synthesize and export silk for spinning cocoons in larvae of Neuroptera and certain Coleoptera (for more details, see RAKITOV in press). In none of these cases have the synthetic products been shown to be related to the excretory function.

Ultrastructural data suggest that brochosomes are unlikely excretory products. The brochosome-secreting cells display only poorly developed basal labyrinth and apical microvilli—cell features that are typically associated with excretion (SMITH & LITTAU 1960; GOURANTON & MAILLET 1967; CHEUNG & PURCELL 1991; RAKITOV 1999a, 2000a). It is unlikely that waste products can be transferred from the other tubule parts into the cells of the glandular segment to acquire here the shape of brochosomes.

3.3 Water-repellence and Incontaminability

Immersing a fresh leafhopper in saline solution to dissect its Malpighian tubules or gluing a leafhopper specimen to a cardboard triangle with a water-based glue are often tedious because the brochosome-coated integument repels the liquids. A simple experiment demonstrates that small droplets of water adhere to clean intact leafhopper integument but are repelled when it is coated with brochosomes (Figs 19-22).

The physical principles of wetting of plant and animal surfaces have been extensively studied (CASSIE & BAXTER 1945; FOGG 1948; HOLDGATE 1955; HOLLOWAY 1970; JUNIPER 1991; WAGNER et al. 1996; BARTHLOTT & NEINHUIS 1997). A commonly used measure of wettability is contact angle (CA), i.e., the angle between the surface and the contour of a small liquid drop placed upon it (Fig. 23A-C). The area of contact between a completely unwettable surface and the drop is infinitesi-

mal (CA=180°). Surfaces are generally referred to as wettable if CA<90° and unwettable if CA>90°. A droplet placed between two solid surfaces (e.g., insect wings) will pull them together if the surfaces are wettable or push them apart if they are unwettable. If any given smooth surface is wettable, adding roughness will increase its wettability. If it is unwettable, the added roughness will increase CA leading to still greater unwettable (for more details, see HOLLOWAY 1970). The materials forming the exposed surfaces in terrestrial plants and insects are often poorly wettable by water due to their chemical properties, but CA on the smooth layers of such materials still does not exceed 110-120° (Fig. 23B). Larger CA (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; BARTHLOTT & NEINHUIS 1997). Roughness can be developed to such an extent that the water-covered surface holds air. Such surfaces, referred to as “composite”, display extremely large CA.

It is clear now that, even if the material comprising the surface of brochosomes is only moderately hydrophobic (due to an external lipid layer? hydrophobic protein chains?), brochosome masses may be highly water-repellent because they form a complex fractal surface structure in the submicron-to-nanometer range (Fig. 12). When covered with water, the layer of brochosomes 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 readily visible on submerged leafhoppers that are coated with brochosomes. Such coats are also repellent to aqueous sugar solutions and other water-based sticky substances (personal observations). Another property of brochosome coats derives from their particulate nature. Because of the loose attachment of the particles to each other and to the integument, a brochosome-coated body part brought in contact with water or a sticky substance will more likely to lose some brochosomes rather than become trapped.

Why should leafhoppers care about wetting and sticking? As with all small terrestrial arthropods, contact with water 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 entomopathogenic fungal spores. Rain and dew endanger insects living far from water. Homoptera, that excrete sticky sugar-containing "honeydew" if feeding on phloem and mesophyll or non-sticky but far more copious watery excretion if feeding on xylem, may be at even greater risk. Not surpris-

duce large amounts of particulate wax that renders them invulnerable to the honeydew (see next section).

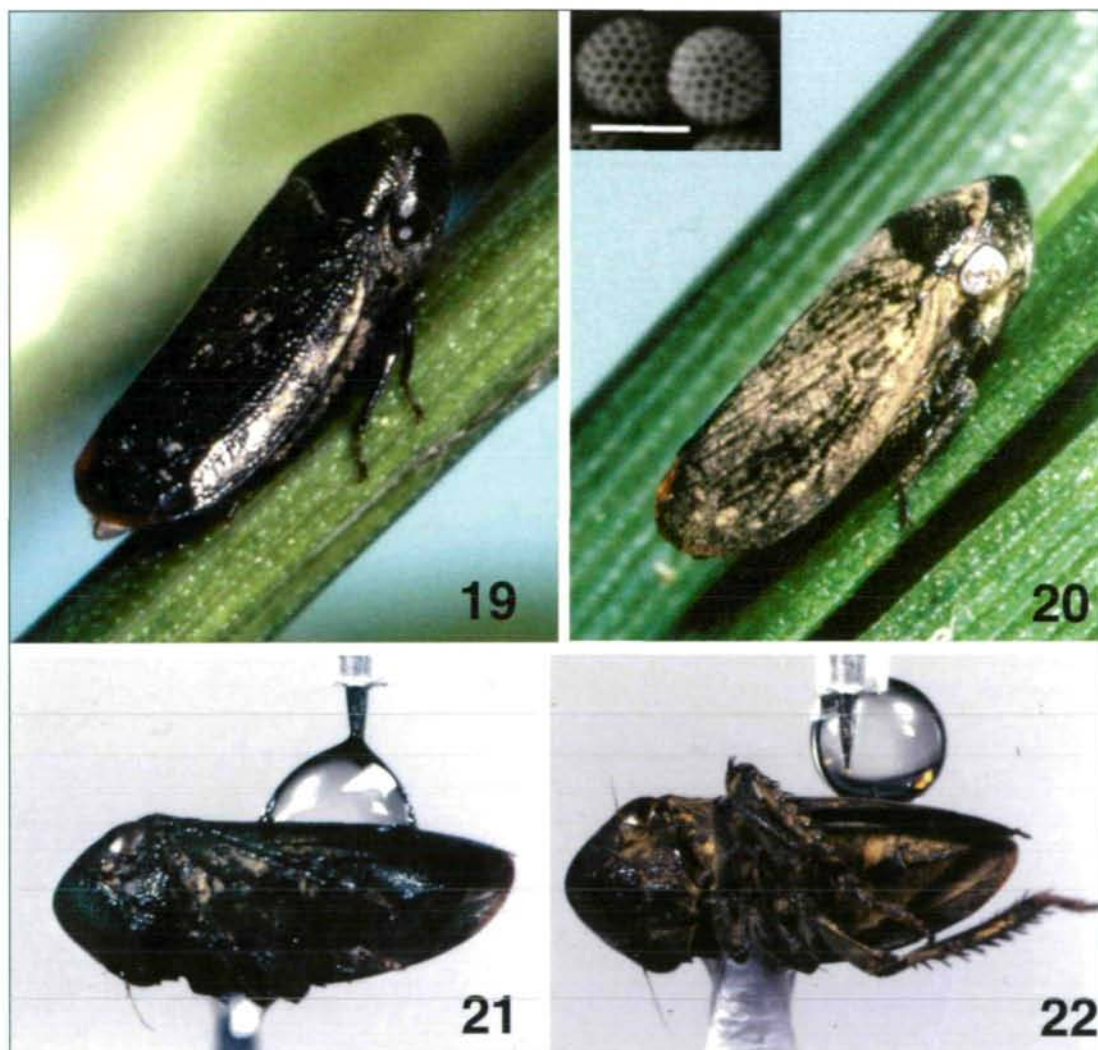
Highly hydrophobic, powdery brochosome coats of leafhoppers may be an efficient protection from rain and dew. The coat may also supplement the excrement-shooting mechanism as a protection from the leafhoppers' own excretion (GÜNTHART 1977; ARZONE

Figs 19-22. Brochosome coat and wettability of the integument in *Nionia palmeri* (VAN DUZEE). Fig. 19. An adult with a poorly developed coat displays its black and shiny cuticle.

Fig. 20. An adult collected at the same locality but at a different time is completely coated with pink brochosome powder. Inset: brochosomes. Bar = 0.5 μ m.

Fig. 21. A droplet of water placed upon the forewing of the specimen from Fig. 19 wets the integument. Contact angle $<90^\circ$.

Fig. 22. A similar droplet does not adhere to the brochosome-coated forewing of the specimen from Fig. 20. Contact angle is ca. 160° .

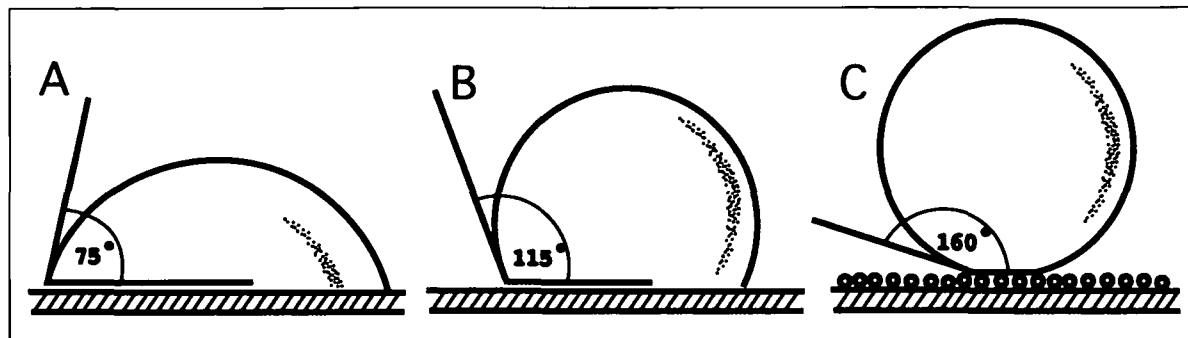


singly, this group demonstrates an array of ingenious defense mechanisms that includes incorporating the excretion into the scale covers in armored scale insects (FOLDI 1982), coating the honeydew droplets with a layer of wax in immature psyllids (WEBER 1930), removing droplets from the anus with the legs in some aphids (KUNKEL 1972), and shooting excreta in aphids, psyllids, whiteflies, and Auchenorrhyncha (WEBER 1930; KUNKEL 1972; STRÜMPFEL 1983). Many Homoptera pro-

duce large amounts of particulate wax that renders them invulnerable to the honeydew (see next section). Highly hydrophobic, powdery brochosome coats of leafhoppers may be an efficient protection from rain and dew. The coat may also supplement the excrement-shooting mechanism as a protection from the leafhoppers' own excretion (GÜNTHART 1977; ARZONE 1986; NAVONE 1987; RARITOV 1995). This kind of protection may be especially important in denser populations, where the leafhoppers are at risk of being contaminated with the neighbor's excrement directly or through the contaminated plant surface. Moreover, the "anal gun" is not completely faultless, and the excretion sometimes accumulates at the rear body end (personal observation). The brochosome coat may prevent the abdomen and wings from sticking together when the excre-

tion penetrates between them or if dew precipitates on their surface. The brochosome powder on the integument may also help leafhoppers escape from spider webs, similarly to lepidopterans 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 spo-

typhlocybinae species in which no brochosomes have been found (*Vilbasteana*, *Aguriahana*, *Kybos* spp.—RAKITOV 1995), in contrast to the brochosome-coated adults, feed on only the lower surfaces of leaves (personal observation). This habit may partly compensate for the absence of the protective particulate coat, because the nymphs are protected from rain and because their honeydew falls down with-



res of certain entomopathogenic fungi (see below). In case of the Proconiini egg nests, the brochosomes placed upon the fresh egg scar, left in the plant by the ovipositor, may prevent flooding of the eggs by plant fluids and ensure the penetration of air to the developing embryos.

Some particular cases of variation in the development of brochosome coats are consistent with these hypothetical functions. Leafhopper species occurring in moist habitats often display a better developed, thicker coat. For instance, *Erotettix cyane* (BOH.), the only Old World leafhopper species known to live on floating leaves, differs from most other Deltocephalinae in being completely coated with a dense, conspicuous layer of brochosomes (hence the species name derived from Gr. *kyanos* = dark blue) and in having permanent reserves of brochosomes in form of spots on the forewings (RAKITOV 1995). In *Scenergates viridis* (VILB.), a deltocephaline that develops inside of closed galls (MITIAEV 1968), the adults are covered with an unusually thick layer of brochosomes (personal observation). The galls contain a "white waxy substance" (MITIAEV 1968), which is probably the brochosomes produced by the nymphs. The importance of protecting the leafhoppers confined in the closed space of the gall from their excrement is obvious. Nymphs of the

out contaminating the leaf surface and the neighbors. Wetting and sticking are especially hazardous for small insects, because such insects may not be able to overcome the surface tension of the liquids. Brochosome coats are usually better developed in smaller leafhoppers. Reserves of brochosomes in the shape of "wax spots" on the forewings are most commonly found in the subfamilies Typhlocybinae (VIDANO & ARZONE 1984) and Xestocephalinae (RAKITOV 2000b), that contain many small species.

3.4 Protection Against Microbes

Several authors listed protection against microbial infection as another possible function of brochosomes (MAYSE 1981; NAVONE 1987; HIX 2001), but this has not yet been tested experimentally. If proved, it may explain the presence of brochosomes on the integument of adult and immature leafhoppers from a wide variety of habitats, as well as on the egg nests.

Like other plant-sucking insects, leafhoppers are not generally susceptible to virus and bacteria pathogens, that need to be ingested to cause infection. However, they are known to be infected by many species of entomopathogenic fungi (SOPER 1985). Most of these appear to be not specific to Cicadellidae. Natural

Fig. 23. Wetting of surfaces displaying different contact angles. (A) Smooth surface of a wettable material; contact angle $< 90^\circ$. (B) Smooth surface of an unwettable material (e.g., wax); contact angle $> 90^\circ$. (C) Surface coated with hydrophobic particles (e.g., brochosomes) holds a film of air and displays the largest contact angle. Redrawn with modification from JUNIPER (1991).

epizootics caused by fungi have been observed in *Cicadella* (MITIAEV 1963), *Cofana* and *Nephotettix* (ROMBACH et al. 1987), *Empoasca* (GALAINI-WRAIGHT et al. 1991), and other leafhoppers (MÜLLER 1956). The entomophthoralean fungus *Zoophthora radicans* (HUMBER et al.) has been studied as a control agent of *Empoasca* (MCGUIRE 1985; WRAIGHT et al. 1990).

Initial events of fungal infection in insects have been reviewed by BOUCIAS & PENDLAND (1991). These 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. Brochosomes may directly protect leafhoppers either by containing substances that inhibit spore germination or kill the germ tubes, or as a mechanical barrier against the spore attachment and subsequent penetration. Because moisture is critical for germination of most pathogenic fungi, brochosomes may also protect leafhoppers indirectly by keeping the integument dry.

Fungistatic lipids and aldehydes have been identified in the cuticle of some insects (SMITH & GRULA 1982; SOSA-GOMEZ et al. 1997). Hypothetically, similar compounds may be present in brochosomes, on the surface or inside. In the latter case, the contents of the particle may be released through the openings in its wall (Fig. 2). In the last instar nymphs of *Empoasca*, most *Z. radicans* penetrations occur through the abdominal intersegmental membranes (MCGUIRE 1985; WRAIGHT et al. 1990). These nymphs have on their integument only a loose coating of brochosomes (micrographs in MCGUIRE 1985). Because the spores of the fungus species known to infect leafhoppers are 3-100 times larger than ca. 0.4 µm brochosomes found in most leafhoppers (SOPER 1985) only a dense layer of brochosomes is likely to keep the spores far enough from the cuticle to prevent their germination. In leafhoppers that display denser brochosome coats, grooming often results in caulking the sutures and intersegmental folds

with brochosomes. Here they may form a significant barrier against spores and germ tubes. The spores trapped in the brochosome coat may be subsequently removed 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 & PENDLAND 1991). As mentioned above, the layer of brochosomes is repellent to sticky substances. In this case, as well as in the case of the pathogenic spores distributed with rain splashes, the brochosome coat may provide efficient protection. In order to germinate and penetrate the host, the spores require moist conditions. Natural epizootics of *Z. radicans* in *Empoasca* coincided with periods of heavy night dews (WRAIGHT et al. 1991). It is not clear whether the spores require presence of films or microdroplets of water on the host integument, but if so, brochosome coats may play a role in protecting leafhoppers from mycopathogens by preventing condensation of water on the body surface.

3.5 Resistance to Desiccation

Another possible role of brochosomes is the protection against desiccation (ARZONE 1986; HIX 2001). Resisting cuticular transpiration is essential for survival of terrestrial arthropods, especially those of small size (HADLEY 1994). It is assumed that the principal barrier to diffusion is formed by the lipids present in the epicuticle. As with certain waxy particulate coats (HADLEY 1979, 1994), brochosomes potentially contribute to the anti-desiccation function of the integument either directly, as a lipid-containing meshwork, or by forming around the integument a boundary layer of unstirred air. Sealing of sutures and intersegmental folds with brochosomes must also reduce water loss. Still another possibility is that brochosomes contain in their central cavities a lipid, that is released through the wall openings (Fig. 2) and forms on the integument an impermeable film. Nevertheless, the ecology of leafhoppers does not suggest that desiccation is a crucial factor for most species. Leafhoppers occur in a variety of habitats, from moist to extremely

dry and, like all plant-sucking insects, receive excessive amounts of water with their food. On the egg nests of Proconiini, brochosomes may play some role in resisting water loss, but such a function also appears unessential because the eggs are protected with the plant epidermis and, most probably, can absorb water from the air and the surrounding living plant tissue, as has been found in other insects (HADLEY 1994). Moreover, the bulk of the Proconiini species that powder their egg nests with brochosomes occur in the wet tropics of the New World (RAKITOV 1999b), where the risk of desiccation is reduced.

3.6 Other Protective Functions

It has been suggested that brochosomes may also serve as a thermal insulation or as a layer reflecting the excessive sunlight, especially in the ultraviolet part of the spectrum (NAVONE 1987; HIX 2001). Brochosome coats are apparently too thin to buffer fluctuations of temperature. However, they indeed reflect UV light (RAKITOV unpublished). This property and the protection from excessive heat may be beneficial for some desert leafhoppers densely coated with brochosomes (e.g. *Achrus ahngerii* (MEL.), Adelungiinae — RAKITOV 1995). Neither excessive sunlight nor heat, however, appear to be principal hazards for the majority of leafhoppers.

Brochosome coats are too thin to mechanically deter predators or parasites. The possibility of chemical deterrence, however, merits future study. As a group, leafhoppers have a large suite of parasites (FREYTAG 1985). Many such species parasitize both leafhoppers and planthoppers occurring in the same habitat (e.g., GREATHEAD 1983; SUBBA RAO 1983). It seems unlikely that such parasitoids have evolved specific mechanisms of overcoming the brochosome barrier and still retained a broad range of the host taxa. More probably, this barrier is not difficult to overcome. The brochosome-coated egg nests of Proconiini are also attacked by numerous parasitoids (TRIAPITSYN et al. 1998; TRIAPITSYN & PHILLIPS 2000).

In some exceptional cases brochosomes may serve as masquerade make-up. *Stenoiorator*

inocarpi BAKER (Nirvaninae) is completely clothed in a conspicuous layer of unusual brick-red brochosomes (personal observation). C. F. Baker, who observed live specimens in Singapore, noticed them first as “little red objects, tightly appressed to the surface of the leaves” which he “passed for some time, supposing them to be scale insects affected by a red parasitic fungus, such affected scales being common in the gardens” (BAKER 1923, p. 378). Further describing the insect, he reports that “it is thickly covered with brick red waxy powder which does not rub off easily” and that the color of the insect is “almost exactly that of the common scale fungus”. This anecdote emphasizes the versatility of the brochosome coats and suggests that disentangling the primary function of brochosomes from their accessory functions may be a difficult task.

3.7 Brochosomes and Pheromones

The unusual and complex shape of brochosomes may be indicative of an equally complex function, such as the accommodation of pheromones (DAY 1993). When inside the secretory cells, brochosomes are filled with an electron-transparent liquid substance (SMITH & LITTAU 1960; GOURANTON & MAILLET 1967; RAKITOV 1999a, 2000a). Apparently the same substance fills the space between brochosomes in the secretory vacuoles. Whether this liquid simply evaporates when brochosomes are placed onto the integument is uncertain. DAY (1993) has suggested that the particles carry a pheromone that slowly evaporates through the openings in the wall compartments (Fig. 2). However, it is unlikely that brochosomes contain a sex pheromone because, in many species, they are produced and applied on the body by the immatures as well as the 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). “Brochosomes-for-eggs” may prevent females from repeated oviposition into the same place (HIX 2001) by releasing a pheromone, but in this situation the visual signal alone would seem to be enough.

4. Brochosomes and Wax: Evolutionary Convergences

4.1 Particulate Waxes

The epicuticle of insects 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. As a modification of this basic process, in some insects, the epidermal glandular cells or their complexes secrete an extracuticular wax in form of particles or filaments. Although such particulate waxes can be found among certain species of Odonata, Neuroptera, Coleoptera, Lepidoptera, or Hymenoptera, they are especially common and diverse in Hemiptera. Especially copious amounts of wax are produced by some Sternorrhyncha and Fulgoromorpha: scale insects (FOLDI 1991), aphids (POPE 1983; SMITH 1999), psyllids (HODKINSON 1974), whiteflies (NAVONE 1987; BYRNE & HADLEY 1988; NELSON et al. 1999), and planthoppers (Figs 24-28) (O'BRIEN & WILSON 1985; MASON et al. 1989). The hemipteran waxes are mixtures that may contain long-chained aldehydes, alcohols, esters of alcohols and fatty acids, free fatty acids, or hydrocarbons, depending on the species and location in the body (MASON et al. 1989; FOLDI 1991; BUCKNER et al. 1999; NELSON et al. 1999). Brochosome coats of leafhoppers have often been confused with extracuticular waxes (VIDANO & ARZONE 1984), and this contributed to the delay in the recognition and study of brochosomes. Brochosomes differ from the epidermal waxes in their genesis, composition, and structure. Nevertheless, both waxes and brochosomes form highly hydrophobic particulate coatings that may have similar functions.

4.2 Waxing Behaviors

The apparent functional analogy between brochosomes and particulate waxes was noted by NAVONE (1987) in his study "Origin, structure, and functions of insect excretions and secretions, of a waxy aspect, distributed on the body by means of legs". Navone has compared the anointing and grooming behaviors of leaf-

hoppers to the behaviors of two unrelated groups of small insects that actively coat their integument with waxy particles: whiteflies (Hemiptera, Aleyrodidae) and dustywings (Neuroptera, Coniopterygidae, also known as "waxflies"). Freshly eclosed whitefly adults scrape off minute chunks of wax from the abdominal fields of wax glands with the legs and distribute them over the entire surface of the body and appendages; the process is assisted by rows of strong setae present on the legs (see also WEBER 1931; BYRNE & HADLEY 1988). Adult dustywings use their legs to make on the integument a coat of microscopic waxy particles produced by scattered groups of epidermal glands. The waxy coats, waxing behaviors and, in the case of whiteflies, leg modifications are remarkably similar to the brochosome coats and related behaviors and structures of leafhoppers. Moreover, the waxy particles, although larger than typical brochosomes (1.3-3.0 μm), also display an intricate surface geometry (NAVONE 1987).

4.3 Functions of Body Wax

The function of the particulate waxy secretions is poorly understood in most instances. Only the waxy blooms of some desert tenebrionid beetles have been studied in some detail from an adaptive perspective (HADLEY 1979; MCCLAIN et al. 1985, 1986; WARD & SEELY 1996). No detailed studies of the properties, except structural and chemical, and function of the hemipteran waxes have been conducted. Functional interpretations of such secretions are mainly derived from comparisons between life histories of the species producing copious wax and those that produce little or no wax. It is remarkable that all of the hypothetical functions proposed for brochosomes have also been proposed for waxes.

Hemipteran waxes have been suggested to be waste products associated with the phytophagy (POLLISTER as cited in POPE 1983, p. 495). Somewhat surprisingly, the possible connection between the nutrition and production of wax in Hemiptera has not been investigated. Some of the long-chained molecules in the waxes are apparently synthesized from smaller subunits by the insects themselves (JACKSON & BLOMQUIST 1976). Still it

remains unclear whether the precursors of such molecules are abundant in plant sap. The fact that in the planthopper family Achilidae the nymphs, feeding on decaying wood or fungi, produce copious wax like the nymphs of other planthoppers (HEPBURN 1967) suggests that feeding on living plant saps is not requisite for wax secretion. Other proposed hypothetical roles of the hemipteran extracuticular waxes include protection from contamination with honeydew and wetting with water, from fungal pathogens, desiccation, parasites and predators, excessive heat, and harmful radiation (HODKINSON 1974; POPE 1983, 1985; NAVONE 1987; BYRNE & HADLEY 1988; FOLDI 1991; SMITH 1999). It has also been suggested that the wax particles produced in whiteflies contain a sex pheromone (BYRNE & HADLEY 1988).

The role of the particulate waxes in protection from honeydew in many cases appears consistent with the biology of the wax-producers. Indeed, the vast majority of the Hemiptera producing copious wax are phloem-feeders excreting sticky honeydew. Some species of Coniopterygidae (but not all) are specialized predators of such hemipterans and may also benefit from a barrier against honeydew. In some aphids and planthoppers, a copious waxy bloom surrounds the anal body end. Excessive production of wax is observed in gall-inhabiting aphids that cannot avoid direct contact with their sticky wastes (SMITH 1999). On the other hand, ant-attended species, from which the honeydew is removed by ants, often produce little wax compared to unattended species. The waxy secretions are extremely hydrophobic (FOLDI 1991; SMITH 1999). As in the case of brochosomes, this may be explained in part by the intricate shape of the wax particles; masses of such particles form a finely sculptured, composite surface capable of holding air. Large accumulations of wax (Fig. 24) may act as a simple mechanical barrier.

In response to low humidity some desert tenebrionid beetles secrete a filamentous waxy bloom that slightly reduces the transpiration rate and is interpreted as a barrier against desiccation (HADLEY 1979). The bloom apparently also plays a role in thermoregulation by reflect-

ing radiation and, in some species, yields a cryptic coloration (MCCLAIN et al. 1985, 1986). 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 & SEELY 1996). The mechanism by which the blooms reduce transpiration is not fully clear. HADLEY (1979) suggested that the actual barriers may be either the meshwork of filaments itself or the layer of unstirred air between these and the cuticle. However, he also pointed out that the filaments eventually "dissolve" into the epicuticular wax and contribute to its thickness. It seems possible that this increase of the epicuticular wax layer at least partly accounts for the observed reduction in water loss, and that the secretion of wax blooms may also be a mechanism to restore this layer that becomes abraded when the insects burrow into the sand. The waxy blooms of Hemiptera may also reduce transpiration from the integument surface, but such a function can hardly explain secretion of wax by a great many species living in mesic or humid environments and receiving an excess of water from plant sap.

4.4 Wax on Eggs

Many insects that produce extracuticular wax use it also to coat their eggs. Examples include whiteflies (WEBER 1931; NAVONE 1987), some aphids (SMITH 1999), and Coniopterygidae (NAVONE 1987). In some whiteflies, the wax that is used to powder the eggs differs in its composition from the body coat wax and is produced by special fields of wax glands that are present in females only (NELSON et al. 1999). In all above cases the eggs are laid exposed. Proposed functions of such wax coatings are the same as for the other life stages: protection from desiccation, flooding, harmful radiation, fungal infection, predators, and parasites. There is a close similarity between the egg laying habits of leafhoppers, that usually insert eggs into plant tissues, and some planthoppers (Fulgoromorpha). Planthoppers, as a group, demonstrate a variety of oviposition techniques and often also coat the egg nests with wax (reviewed in O'BRIEN & WILSON 1985). Some species insert eggs into living plants and powder the egg scar with wax. This behavior closely resembles the ovi-

position behavior of the Proconiini leafhoppers that powder their egg nests with brochosomes (Figs 15-18). In Illinois, I observed females of *Stenocranus* sp. (Delphacidae) laying groups of several eggs into the blades of *Cyperus esculentus* L. After inserting each egg the female brushed the ventral side of the abdomen tip against the plant, rubbing the copious wax produced on the pygofer into the

egg scar and the surrounding epidermis (Fig. 25). The completed egg nests were conspicuous due to the powdery coats (Fig. 26), that consisted of broken wax filaments with a highly complex surface structure (Figs 27-28). In another delphacid species, to place wax onto the egg scars, females use their hindlegs (METCALFE 1968). As with the brochosome coats of egg nests, the function of these coatings may

Figs 24-28. Secretion and use of wax in planthoppers.

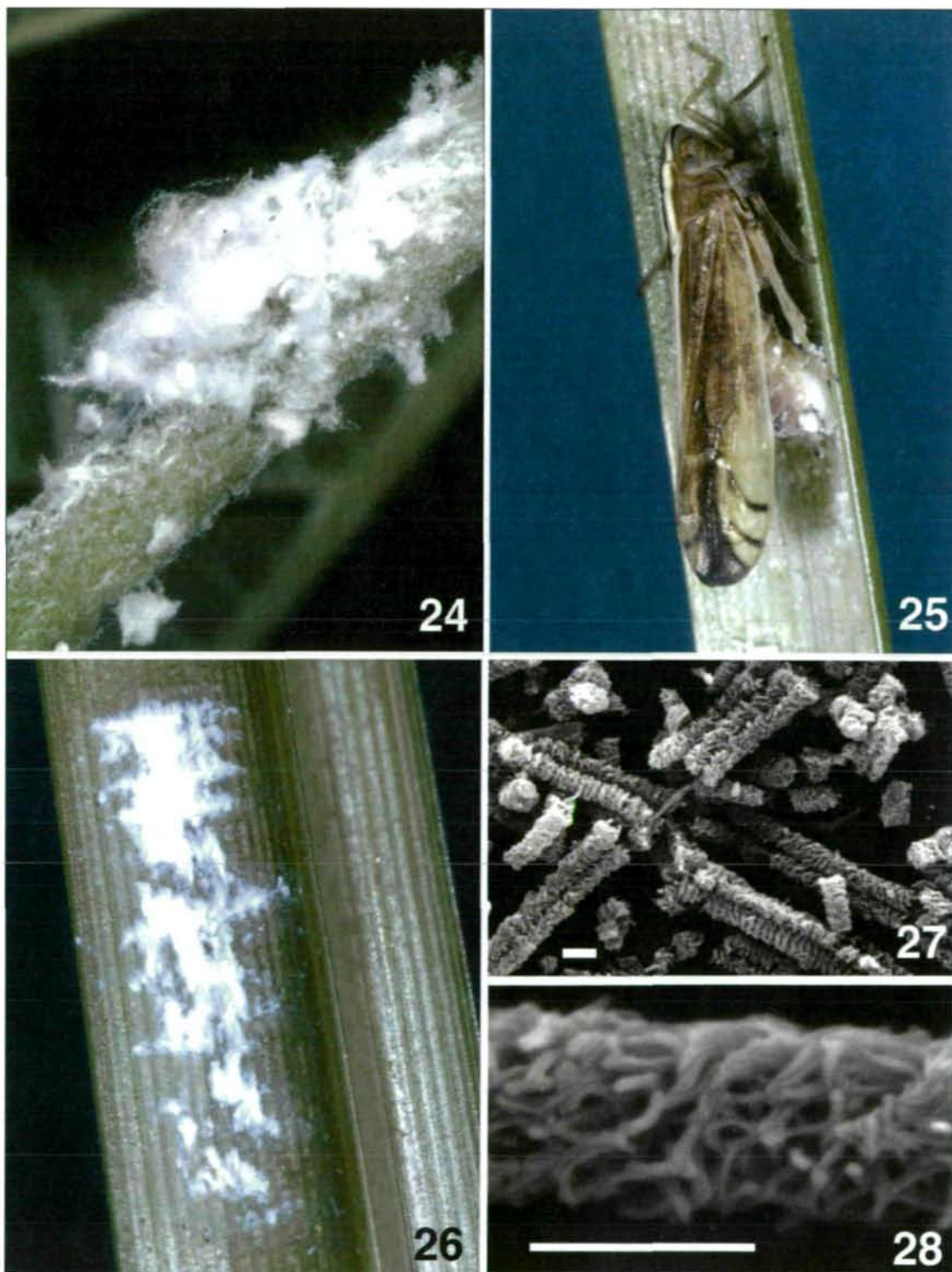
Fig. 24. A 5th instar nymph of *Anormenis* sp. (Flatidae) is barely visible under the "cotton candy" of wax it has produced. The wax also covers the nearby parts of the host plant stem.

Fig. 25. Female *Stenocranus* sp. (Delphacidae) coating her eggs, inserted into a blade of *Cyperus*, with the wax secreted on its pygofer. She is rubbing the ventral surface of the abdomen end, where a white mass of wax is visible, against the egg scar and the surrounding plant epidermis.

Fig. 26. The completed oviposition site of *Stenocranus* sp. The eggs are not visible under the plant epidermis and wax.

Fig. 27. Rods of wax from the egg nest of *Stenocranus* sp. Bar = 1µm.

Fig. 28. Close-up of one such rod reveals an extraordinarily complex surface structure. Bar = 1µm.



be the protection of the egg scar from flooding with plant fluids (to aerate the eggs) and the protection against infection of the wound with phyto- and entomopathogenic microbes. Remarkably, in both Cicadellidae and Delphacidae only a few species coat their egg nests with the particulate materials. Further comparative studies of the ecology of oviposition and, perhaps, of the respiratory systems of the eggs in the two families may shed light upon the adaptive significance of these unusual maternal behaviors.

4.5 Leafhoppers: Brochosomes Instead of Wax?

Among Cicadomorpha, conspicuous amounts of particulate body wax are produced in cicadas (Cicadoidea) and certain treehoppers (Membracidae). 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 brochosomes and waxes have a similar function. At the same time, small amounts of particulate wax have been found in some leafhopper species (RAKITOV 1995). In particular, the integuments display a thin particulate waxy coating in the nymphs of certain species from Macropsinae, Idiocerinae, Typhlocybinae, and in nymphs and adults of *Iassus*, all of which apparently do not produce brochosomes. Very small amounts of wax have also been found on certain body parts of several species known to produce brochosomes (e.g., in *Oncometopia*, *Aphrodes*, *Doratura*), suggesting that this secretion can sometimes coexist with brochosomes. Further studies will probably find microscopic quantities of the particulate wax in more leafhopper species. The incidental coexistence of brochosomes and wax does not necessarily dismantle the hypotheses of functional similarity between these secretory products. It should be noted, however, that the apparent absence of wax in leafhoppers may have explanations other than substitution with brochosomes. The absence of massive waxy blooms (as in Fig. 24) in leafhoppers is clearly related to their agility: leafhoppers can escape certain hazards actively instead of relying on the passive protection.

5. Brochosomes and Evolution of Cicadellidae

Brochosomes are a tentative apomorphy of Cicadellidae (DIETRICH et al. 2001). Correlating the origin of the family with certain shifts in the life history or habitat may shed light upon the primary function of brochosomes. Relationships between the extant leafhopper taxa that display variation in the brochosome-related traits may also provide necessary clues.

Evolution of the major cicadomorphan lineages—Cercopoidea (spittlebugs), Cicadoidea (cicadas), and Membracoidea (leafhoppers and treehoppers)—has been reviewed by DIETRICH (this volume). Synthetic modifications of the Malpighian tubules are characteristic of all the three lineages (RAKITOV in press). In spittlebugs and cicadas, the tubules synthesize and export secretory products during the nymphal stage only. Such products are released with the main flow of excreta. In spittlebugs they make a part of the nymphal “spittle”, and in cicadas a part of the “anal liquid” that is used to strengthen the walls of the nymphal burrows and clean the body. Production of secretory products in the Malpighian tubules and their application on the integument may, therefore, be plesiomorphic traits of Cicadomorpha. I have conjectured (RAKITOV in press) that the nymphs of early cicadomorphans were subterranean and xylem-feeding, 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. The integument of such nymphs was coated with their own liquid excreta and secretions, as in modern spittlebugs. In the lineage leading to the extant Membracoidea, the nymphs switched from cryptobiosis to the open and agile life style. Apparently, they retained the habit of applying the Malpighian tubule products on the integument instead of developing protective extracuticular waxy coatings. Anointing behavior, therefore, may have evolved before the origin of brochosomes, and its original function might have been different from that in extant leafhoppers. Brochosomes or their precursors are not known in

the fossil record. However, the imprints of the earliest known membracoids, Karajassidae from the Jurassic (SHCHERBAKOV 1992), show rows of tibial macrosetae that may have served for distribution of a particulate material over integument, as in modern Cicadellidae (RAKITOV 1998).

Among modern Cicadellidae, brochosomes have been found in all of the major subfamilies (RAKITOV 1995, 1998), suggesting that extant "pre-brochosomic" leafhoppers may not exist. Xylem-feeding, displayed among the extant leafhoppers by Cicadellinae (*sensu lato*) only, is considered to be a plesiomorphic trait shared with cicadas and spittlebugs (e.g., HAMILTON 1983). Such a diet results in production of copious watery, non-sticky excreta. The fact that Cicadellinae produce brochosomes suggests that this secretion evolved before the more advanced leafhopper lineages switched to phloem-feeding and, therefore, that protection from sticky honeydew could not be its original function. Relationships among the extant Cicadellidae are only partly resolved (DIETRICH et al. 2001). However, the subfamilies that contain species not producing brochosomes at the nymphal stage (Macropsinae, Idiocerinae, Ulopinae, and Typhlocybinae) appear to be advanced lineages, suggesting that production of brochosomes during both the nymphal and adult stages is a plesiomorphic trait.

The variation of brochosome structure among leafhoppers gives little indication of evolutionary trends. One type of brochosomes, 0.3-0.6 μm spherical honeycombs found in the majority of studied species from a variety of subfamilies (Fig. 2) (RAKITOV 1995, 1999a), may represent a plesiomorphic type. "Brochosomes-for-eggs", produced by females of certain Proconiini genera, are usually elongate and significantly larger than "brochosomes-for-integument" (Fig. 18) (RAKITOV 1999b and unpublished; HIX 2001). These properties may facilitate storing the particles on the forewings and transferring them onto the egg nests. Masses of larger particles may also ensure better access of air to the eggs. At the earlier stages, the same females produce and apply onto the body typical "brochosomes-for-integument" (RAKITOV 2000a; HIX

2001). This clearly indicates that the use of brochosomes in oviposition is a derived trait. Preliminary results suggest that the egg nest powdering is an apomorphy of a single lineage within Proconiini that contains ca. 200 described species (RAKITOV 1999b).

Treehoppers (Aetalionidae, Melizoderidae, and Membracidae) have apparently arisen from within Cicadellidae and lost the capability of synthesizing brochosomes secondarily (RAKITOV 1998; DIETRICH et al. 2001). Nymphs of Membracidae share a specific type of anointing—"bathing" in the brochosome-free Malpighian tubule secretions—with nymphs from the subfamilies Macropsinae, Ulopinae, and Agalliinae that represent a sister lineage of treehoppers (RAKITOV 1996; DIETRICH et al. 2001). This indicates that during the evolution of treehoppers the production of brochosomes was first lost by the immatures and later by the adults. These changes may be related to ant-mutualism, that is frequently observed among plesiomorphic treehoppers and sometimes in the related subfamilies of leafhoppers (DIETRICH this volume). Other life-history traits of treehoppers that may correlate with the absence of a brochosome coat are the sedentary life style, gregariousness, and parental care.

6. Conclusion and Perspectives

Available evidence suggests that the most likely role of brochosomes in Cicadellidae is that of a protective coating that (1) repels water and sticky honeydew and/or (2) prevents fungal infection. These hypothetical functions are generally consistent with the biology of leafhoppers and at least partly related to the peculiar structure of brochosomes. Similar functions are performed in other insects by the epidermis and its derivatives, in particular, waxes. The apparent functional substitution of epidermal waxes with the Malpighian tubule secretions may have been associated with the cryptic life mode of the immatures in early Cicadomorpha and related physiological and behavioral constraints. Inadequate knowledge of the properties of brochosomes makes these functional interpretations highly speculative.

A variety of approaches may contribute to defining the function of brochosomes. First, biochemical studies of brochosomes are needed to clarify the relation between this secretion and the metabolism of leafhoppers. They would also provide additional hints regarding the function of brochosomes. Brochosomes need to be isolated, purified, and studied by modern analytical methods, and their composition should be compared between species as well as between "brochosomes-for-integument" and "brochosomes-for-eggs". Of special interest is the inner contents of the particles because, as was pointed out by DAY (1993), the secret of brochosomes may be in their filling. In addition, the nature of the alternative Malpighian tubule products, found in certain leafhopper nymphs and in Membracidae, should be studied to clarify the relationship between such products and brochosomes, and to elucidate the function of the brochosome-free anointing behaviors. Anti-microbial properties of brochosomes also need to be tested. Such tests should employ a representative sample of known fungal pathogens of Hemiptera, including species with different modes of distribution and attachment of propagules (SOPER 1985; BOUCIAS & PENLAND 1991). In the design of these experiments, special care should be taken to separate potential direct and indirect effects of brochosomes. Wettability, contaminability, and other surface properties of brochosome layers also await experimental study. In particular, it would be interesting to know which physical properties correlate with the variation in size, shape, and sculpture of brochosomes observed among leafhopper species. As with the microbiological assays, such studies may be conducted on intact leafhoppers, denuded "brochosome-free" leafhoppers (obtained by impeding anointing behavior, RAKITOV unpublished), and substrates artificially coated with brochosomes. Valuable insights into the function of brochosomes can also be derived from behavioral observations. Of particular interest are the frequency and duration of anointing and grooming in relation to certain environmental parameters, e.g., humidity. Laboratory studies of these behaviors may help explain individual variation in the development of the bro-

chosome coat (e.g., in *Nionia*, Figs 19, 20). Other insights may be gained by study of the brochosome coats of the leafhopper species inhabiting contrasting environments. Such studies should take phylogeny into account to correct for its effects on the distribution of the observed characters (e.g., WARD & SEELY 1996). Phylogenetic methods will also be instrumental in elucidating the causes of secondary losses of the brochosome production, that apparently occurred independently multiple times. Development of techniques for quantification of brochosomes on the body of individual leafhoppers will be important for all kinds of functional studies. Comparative ultrastructural studies are needed to find out whether the presence of brochosome coats in leafhoppers correlates with some modifications of their epicuticle. Finally, studies of brochosomes and related aspects of the leafhopper biology should be extended to additional taxa, including little-known groups, particularly Myerslopiidae, thought to be the most plesiomorphic family of the extant Membracoidea (HAMILTON 1999).

Future studies may uncover functions of brochosomes different from any of the hypothetical roles discussed above. One of the challenges for such studies is the apparent multifunctionality of the brochosome coats, characteristic of all structures that form an interface between an organism and the environment. Testing and confirming any of the functions discussed above may not be sufficient to conclude that it is the primary and original function of brochosomes. Therefore, functional interpretation of brochosomes will only be possible within a broader context of comparative and experimental studies.

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7. Zusammenfassung

Brochosomen sind rätselhafte Strukturen, die ausschließlich bei Zwergzikaden (Cicadellidae) zu finden sind. Es handelt sich um ultramikroskopisch kleine Protein-Lipid-Partikel, die in speziellen Zellen der Malphigischen Organe produziert werden. Zwergzikaden verteilen die über die Analöffnung ausgeschiedenen Brochosomen mit ihren Beinen aktiv auf der gesamten Körperoberfläche und manchmal auch auf Eigelegen. Die geringe Größe und die komplexe Oberflächenstruktur der Brochosomen bedingt eine Unbenetzbarkeit von Brochosomensichten sowohl für Wasser als auch für die klebrigen, zuckerhaltigen Exkremente der Zikaden. Eine weiterer möglicher Zweck der Brochosomensichten ist ein direkter oder indirekter Schutz vor Pilzsporen und -infektionen. Andere in der Literatur genannte Funktionen wie Schutz vor Austrocknung, UV-Strahlung, Thermoisolation und Schutz vor Freßfeinden und Parasiten spielen vermutlich hingegen - wenn überhaupt - nur eine untergeordnete Rolle. Keine dieser genannten Eigenschaften ist bisher experimentell überprüft worden. Dennoch ist anzunehmen, daß Brochosomen die grundsätzlich gleichen Funktionen wie epidermale Wachsüberzüge der Körperoberfläche und der Eier verschiedener anderer Insekten erfüllen.

Die Synthese dieser Sekrete der Malphigischen Organe und die Methode der Verteilung auf die Körperoberfläche könnten bei frühen Cicadomorpha zunächst als Anpassung an eine unterirdische Lebensweise der Larven entstanden sein. Beim Übergang zur oberirdischen Lebensweise könnten diese Merkmale dann Schutzfunktionen übernommen haben, die bei anderen Insekten von epidermalen Wachsen erfüllt werden. Aufgrund unzureichende Kenntnisse der Eigenschaften von Brochosomen und des völligen Fehlens entsprechender experimenteller Studien sind derartige Aussagen gegenwärtig allerdings spekulativ. Um die Bedeutung der Brochosomen endgültig klären zu können, wären umfassende vergleichend-experimentelle Studien wünschenswert.

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Address of the author:

Dr. Roman RAKITOV
Illinois Natural History Survey
607 E. Peabody Dr.
Champaign, IL 61820 U.S.A.
E-mail: rakitov@inhs.uiuc.edu

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