

**Post-moulting behaviour associated with Malpighian tubule secretions
in leafhoppers and treehoppers (Auchenorrhyncha: Membracoidea)**

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**Behaviour, physiology, phylogeny, Auchenorrhyncha, Cicadellidae, Membracidae, anointing,
grooming, brochosomes, Malpighian tubules, secretion**

Abstract. The acts of excreting special fluids containing the secretory products of the Malpighian tubules and smearing them onto the cuticle, referred to as anointing, were observed after moult in 12 species of subfamilies Cicadellinae, Typhlocybinae, Iassinae, Deltocephalinae, Idiocerinae, Macropsinae, Agallinae, Ulopinae (Cicadellidae) and in *Gargara genistae* (F.) (Membracidae). Among the species studied anointing was not recorded in Idiocerinae nymphs and in adults of *Iassus lanio* (L.) only. Usually the first act of anointing was observed 1–3 hours after moulting. Nymphs of *Oncopsis*, *Macropsis*, *Agallia*, *Ulopa* and *Gargara* bathe in secretory drops excreted onto the plant surface, in contrast to other nymphs exuding fluids directly onto the body. The fluids excreted by adult leafhoppers of all subfamilies and by nymphs of some Deltocephalinae and *Cicadella* were found to contain brochosomes, secretory products of the specialized Malpighian tubules. In these cases anointing is associated with repetitive acts of grooming, resulting in formation of a coating of brochosomes on the cuticle. In contrast, the nymphs of the other leafhopper subfamilies, as well as nymphs and adults of *G. genistae*, secrete brochosome-free fluids, which appear distinct from faeces and contain other secretory products, which, probably, originated from the Malpighian tubules. In addition, nymphs and adults of various leafhopper species occasionally wash their tarsi with a transparent fluid (probably faeces) secreted from the anus. The functions of the brochosome-free anointing and the washing of legs remain obscure. Nevertheless, the characters related to anointing appear to be of value for the classification and assessing relationships between the higher taxa of the Membracoidea.

INTRODUCTION

Leafhoppers (Cicadellidae) and treehoppers (Membracidae) excrete, in addition to faeces, special fluids containing the products of the Malpighian tubules and apply them to the body surface. These curious rituals, which are not found in other insects, occur regularly after moulting, and they are referred to as post-moult anointing.

The first example of such behaviour was described by Storey & Nichols (1937) as the “second method of defaecation” in the leafhopper, *Cicadulina mbila* Naude (Deltocephalinae): the adult secretes several droplets of opaque fluid from the anus, transfers them with its hind legs onto the tegmina and, as soon as the secretion hardens, grooms itself violently. It has been recognized later that, in this way, leafhoppers cover their cuticle with so-called brochosomes (BS) – minute spherules (as a rule 0.5 µm in diameter) with a characteristic sculpture (Fig. 24), made up from lipids and proteins and produced by highly specialized Malpighian tubules of the Cicadellidae (Tulloch et al., 1952; Day & Briggs, 1958; Smith & Littau, 1960; Gouranton & Maillet, 1967; Arzone, 1986; Cheung &

Purcell, 1991; Day, 1993). Using their legs leafhoppers distribute BS over the cuticle making a covering, found in all the major subfamilies of the Cicadellidae (Rakitov, 1995). It is possible that the brochosomal covering serves as a form of protection, like wax coats in other insects (Günthart, 1977; Arzone, 1986; Navone, 1987; Rakitov, 1993, 1995), but neither this nor other possible functions of BS have been tested and verified experimentally. The characteristic structure of legs in the Cicadellidae is clearly associated with the distribution of BS over the cuticle showing key role of this specialization in origin and evolution of the family (Rakitov, 1993). There is some contradictory evidence for the production of BS in the Membracidae. Gouranton & Maillet (1967) and Day (1993) reported they have found BS in some treehoppers, but gave no further details. However, no BS were found in various treehoppers studied by scanning electron microscopy by Dietrich (1989) and the present author (unpubl.).

The secretion of BS, anointing and related phenomena were not studied adequately. Although the acts of anointing can easily be observed, only few descriptions of this behaviour in the Cicadellidae have been made in addition to that of Storey & Nichols (1937). After the short report of Vidano & Arzone (1984) on anointing in adult *Zyginidia pullula* (Boheman), Navone (1987) described it thoroughly in nymphs and adults of *Z. pullula*, as well as in adults of *Rhytidodus decimusquartus* (Schrank). Similar observations have been made in *Vilbasteana oculata* (Lindberg) (Rakitov, 1992). Thus, anointing has been described only in 4 species representing 3 leafhopper subfamilies. As far as is known, no similar behaviour was reported for non-cicadellid Membracoidea.

The purpose of this study was to examine anointing in major subfamilies of Palaearctic leafhoppers and treehoppers.

MATERIAL AND METHODS

The nymphs were collected in Moscow and Moscow region: *Cicadella viridis* (L.) from sedges; *Vilbasteana oculata* (Lindberg) from lilac; *Cicadula quadrinotata* (F.), *Graphocraerus ventralis* (Fallen) and *Balclutha punctata* (F.) from sedges and grasses; *Iassus lanius* (L.) from oak; *Idiocerus stigmatalis* Lewis from willow; *Populicerus nitidissimus* (Herrich-Schäffer) and *Macropsis graminea* (F.) from black poplar; *Oncopsis flavicollis* (L.) from birch; *Agallia venosa* (Fourcroy) from maiden pink; *Ulopa reticulata* (F.) from heather and *Gargara genistae* (F.) from broom. Insects were reared in the laboratory (temperature 25–27°C, RH 60–75%) on the cut twigs of their host-plants from several hours to several weeks. Behaviour of freshly moulted nymphs and adults was closely observed under the stereomicroscope. Figures were drawn from photographs and colour slides taken with a camera supplied with an attachment for macrophotography and flash. To ascertain the presence of secretory particles, samples of fluids were taken at the instant of emission with a glass capillary and transferred directly onto the stubs for scanning electron microscopy, and allowed to dry. Spots of hardened secretion as well as dry specimens and exuviae glued to the stubs were coated with platinum-palladium alloy and examined using a Hitachi 405S scanning electron microscope. In addition, extirpated Malpighian tubules of nymphs of Macropsinae were crushed on the stubs and examined in the same manner. The highest 30,000 × magnification permitted viewing of particles as small as 0.15 µm in diameter.

RESULTS

The main features of the acts of anointing described below are summarized in Table 1.

TABLE 1. Main features of the observed acts of anointing, 2-step smearing, smearing and bathing designate different types of the acts (see text for details). Absence (–) or presence (+) of the typical acts of grooming refer to the whole period of observation; – + indicates scarce grooming movements. I – adult, N – nymph (numbers indicate instars), BS – brochosomes.

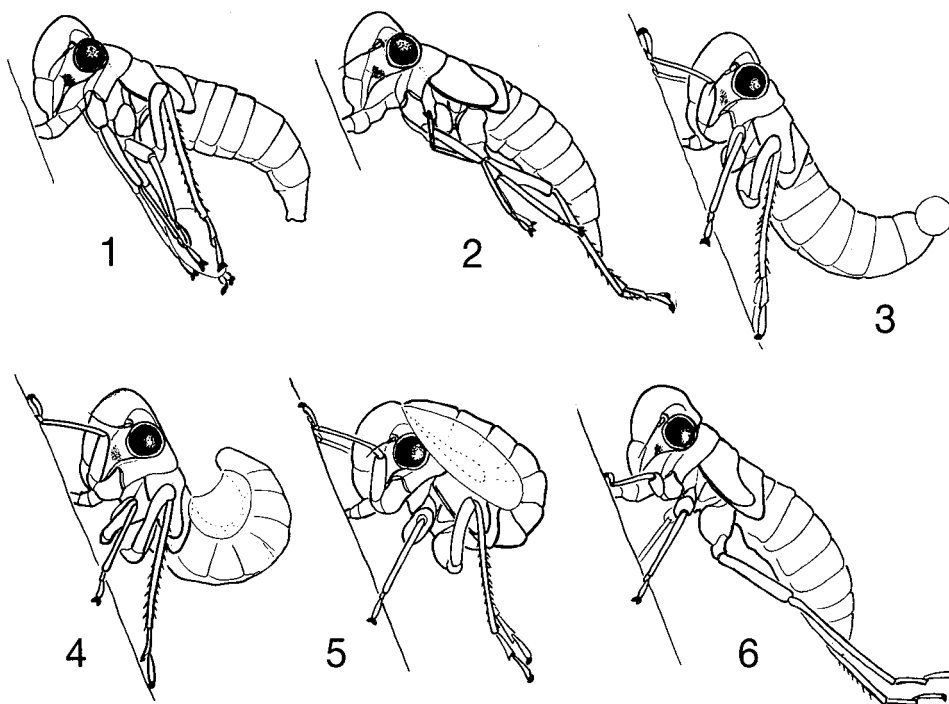
Taxon	Stage	Act of anointing	Grooming	Particles in secretion
CICADELLIDAE				
Cicadellinae				
<i>Cicadella viridis</i>	N4, N5	2-step smearing	+	BS
	I	2-step smearing	+	BS
Typhlocybinae				
<i>Vilbasteana oculata</i>	N5	2-step smearing	–	no
	I	smearing	+	BS
Deltocephalinae				
<i>Cicadula quadrinotata</i>	N5	2-step smearing	+	BS
<i>Graphocraerus ventralis</i>	N3	2-step smearing	+	BS
<i>Balclutha punctata</i>	I	2-step smearing and smearing	+	BS
Iassinae				
<i>Iassus lanio</i>	N5	2-step smearing	–	no
	I	no	– +	
Idiocerinae				
<i>Idiocerus stigmatalis</i>	N1-5	no	–	
<i>Populicerus nitidissimus</i>	N1-5	no	–	
	I	smearing	+	BS
Macropsinae				
<i>Oncopsis flavicollis</i>	N4, 5	bathing	–	spheroids
	I	smearing	+	BS
<i>Macropsis graminea</i>	N4, 5	bathing	–	spindle-like particles
	I	smearing	+	BS
Agalliinae				
<i>Agallia venosa</i>	N5	bathing	–	no
Ulopinae				
<i>Ulopa reticulata</i>	N5	bathing	–	no
	I	2-step smearing	–	BS
MEMBRACIDAE				
Centrotinae				
<i>Gargara genistae</i>	N2-5	bathing	–	no
	I	smearing	– +	no

Family Cicadellidae
Subfamily Cicadellinae
Cicadella viridis (L., 1758)

Nymph

Anointing was observed in one 4th instar nymph roughly 130 min after moulting and in one 5th instar nymph (the time was not recorded). The two steps involved in the process are as follows:

1) The nymph, using both hind legs, picks up the single drop of the fluid exuded from the rear end and then smears it by kneading movements of all the legs onto the legs and the ventral part of the body (Figs 1, 2). When doing so on the upper surface of the plant the



Figs 1–6: *Cicadella viridis*, 4th instar nymph. 1, 2 – first step of anointing; 3–6 – second step.

nymph balances itself on its rostrum and face, whereas on the underside it hangs by its beak alone. Thereupon, the insect returns to its normal posture and rests.

2) About 5 min later the second drop appears from the rear end (Fig. 3). Now the insect curves the tip of the abdomen upward and forward so that the fluid is spread on the dorsal surface of the body (Fig. 4). Eventually the abdomen covers the thorax and head completely (Fig. 5). In this position the insect spreads with its fore legs liquid from the edge of the drop onto the eyes and face. Then it adopts the normal posture, being almost moist throughout, and rests until the secretion dries. Thereupon, the nymph starts to groom itself violently (Fig. 6).

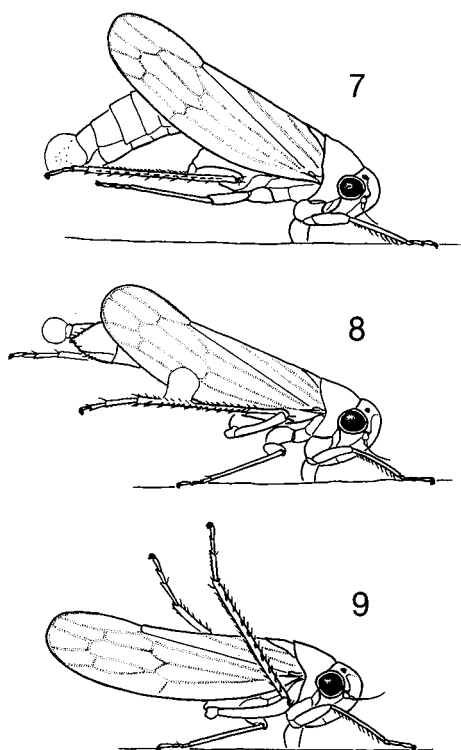
SECRETION. The fluid is white opaque, opalescent and leaves a white sediment consisting of BS.

Adult

The following two-step pattern was observed in one freshly moulted adult (the time was not recorded):

1) The leafhopper secretes from the rear end the single drop of fluid (Fig. 7) and smears it onto the legs and ventral parts of the body, as in the nymph.

2) Several minutes later the insect raises the abdomen, curves its tip slightly downwards and secretes 4 opaque droplets sequentially, catching them alternately by the left and right hind tibiae which transfer each droplet to the costal area of the corresponding tegmen (Fig.



Figs 7-9: *Cicadella viridis*, adult. 7 – beginning of the first step of anointing; 8 – second step; 9 – grooming.

(Rakitov, 1992). Up to 4 acts of anointing occur during the first 4 h after moult. A brief outline of this behaviour is given here.

Some 60–120 min after moulting 4 secretory droplets are exuded and transferred onto the special rugose areas near the middle of the anterior margin of the tegmen, specific to the Typhlocybinae, where the fluid hardens as white spots, one on each wing (so called wax-areas). Thereafter the insect distributes this dry material over the whole cuticle in repetitive acts of grooming.

SECRETION. The fluid is light pink, opaque, opalescent and leaves a white sediment consisting of BS.

NOTES. 1) In one individual of *V. oculata* at the age of about a month the wax-area spot was artificially removed, but the next day it reappeared. Therefore, adults retain the ability to emit the secretion during their lifetime.

2) Some observations on anointing in Typhlocybinae may be added. In *Alebra wahlbergi* (Boheman) and *Eurhadina untica* Dlabola, in the first act of anointing, the insects press their hind tibiae bearing the droplets to the costal area of the tegmina until the secretion dries. As a result, two elongated irregularly-shaped spots are formed, and they

8). Then the tibiae spread the fluid over the entire surface of the tegmina. Crossed tibiae push the fluid forward to the scutellum and pronotum where the fore legs pick it up and smear it onto the head and even rostrum. As soon as the secretion hardens the grooming begins (Fig. 9).

SECRETION. The fluid is white opaque, opalescent and leaves a white sediment consisting of BS.

Subfamily Typhlocybinae *Vilbasteana oculata* (Lindberg, 1929)

Nymph

In a previous paper (Rakitov, 1992) I reported by mistake that this species never exhibits anointing at the nymphal stage. Later, a two-step anointing process, identical to that of the *Cicadella* nymph, was observed in two 5th instar nymphs 130 min after moult. No grooming was recorded thereafter.

SECRETION. The fluid is only slightly white and opalescent, it leaves no visible sediment. No particles were found therein by SEM examination.

Adult

The behaviour, observed in about fifty leafhoppers, has been described in detail

disappear during the next act of grooming. In the second act of anointing, occurring a short time later, the tibiae are removed from the tegmina as soon as the fluid is transferred. The drops of fluid dry as oval wax-area spots. In contrast with *V. oculata*, *E. untica* emits only two drops of secretion: one for each tegmen.

Subfamily Deltocephalinae

Nymph

Two-step acts of anointing, similar to those of the *Cicadella* nymph, were observed in one 5th instar nymph of *Cicadula quadrinotata* (F.) and in one 3rd instar nymph of *Graphocraerus ventralis* (Fallen) about 60 min after moult. After anointing the nymphs start grooming.

SECRETION. The fluid is white opaque, opalescent and leaves a white sediment consisting of BS.

Adult

Two successive acts of anointing were observed in two *Balclutha punctata* (F.) adults (the time was not recorded). The first act is comprised of two steps closely similar to those of the *Cicadella* adult. The insect smears the first drop of fluid onto the legs and ventral parts of the body, and about 5 min later it smears other 4 drops onto the entire upper surface of the tegmina. Then the insect grooms itself violently. About an hour after that the next act of anointing occurs. In this case the tibiae only transfer secretory droplets onto the tegmina where they harden as two elongated wax-area spots. These spots fade away completely in subsequent acts of grooming.

SECRETION. The fluid is white opaque, opalescent and leaves a white sediment consisting of BS.

Subfamily Iassinae

Iassus lanio (L., 1761)

Nymph

Two-step anointing, as in nymphs of *Cicadella*, was observed in the 5th instar nymph, 170 min after moulting. No grooming was recorded thereafter.

SECRETION. The fluid is only slightly opalescent and leaves no visible sediment. No particles were found after examination by SEM.

Adult

No anointing was recorded in three individuals observed after moulting. Sometimes irregular grooming movements occurred.

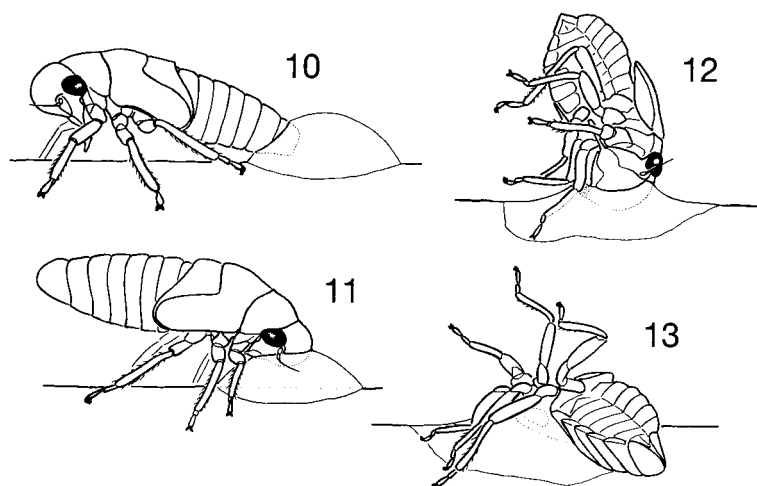
Subfamily Idiocerinae

Populicerus nitidissimus (Herrich-Schäffer, 1835)

Nymph

No acts of anointing were recorded in nymphs of different instars.

NOTE. The same was found in nymphs of *Idiocerus stigmatalis* Lewis.



Figs 10–13: *Oncopsis flavicollis*, 5th instar nymph. Anointing.

Adult

In one individual the anointing was recorded in 165 min after moulting. The behavioural pattern is similar to that in the second step of adult *Cicadella*. Hind tibiae spread the secretory droplets over the tegmina. Crossed tibiae push the fluid forward to the scutellum and pronotum, where fore legs pick it up and smear it onto the head. As soon as the secretion dries, the insect starts grooming.

SECRETION. The fluid is white opaque, opalescent and leaves a white sediment consisting of BS.

Subfamily Macropsinae *Oncopsis flavicollis* (L., 1761)

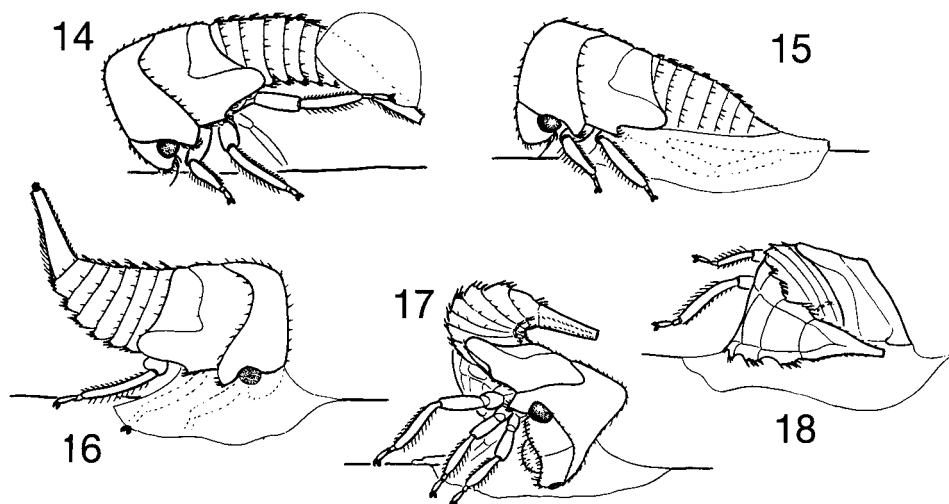
Nymph

A peculiar type of anointing, referred to as bathing, was observed in four 4th instar and five 5th instar nymphs 90–180 min after moult.

The resting nymph suddenly starts to crawl and a few seconds later it exudes a single drop of fluid onto the plant surface (Fig. 10). Thereupon, it turns around and dips its head into the drop (Fig. 11). Later the nymph lays down on its side into the fluid and turns onto its back (Figs 12, 13). This sequence of events recurs several times until the whole drop is spread onto the body. As a result, the entire surface of the cuticle becomes wet with the secretion. Thereafter, the insect rests and dries. No grooming is observed.

Bathing occurs on the upper side as well as on the underside of the branch, petiole or leaf. In the latter case, the viscosity of the fluid alone prevents the overturning nymph from falling.

SECRETION. The fluid is only slightly opalescent and white, and leaves no visible sediment on the cuticle (a thin light deposition can be seen when the fluid is placed onto the glass.). Examination by SEM found that it contained only spherical particles of 0.6–1.7 μm



Figs 14–18: *Gargara genistae*, 5th instar nymph. Anointing.

in diameter with rough surface (Figs 22, 23). The same particles were found in the Malpighian tubules and on the cuticle of nymphs. Spheroids are scattered all over the cuticle and do not form a continuous coating.

Adult

Anointing was observed in one adult 180 min after moulting; in two others the time was not recorded. In general, the process resembles the second step of the act of anointing in adult *Cicadella*. 4–7 secretory droplets are transferred onto the tegmina and spread by the hind tibiae. The pygofer and the ventral side of the abdomen can also be rubbed by wet hind legs. After the secretion dries the insect starts grooming.

SECRETION. The white opaque, opalescent fluid leaves a white sediment consisting of BS (Fig. 24).

Macropsis graminea (F., 1798)

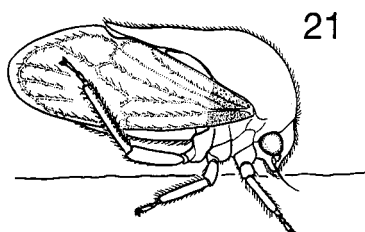
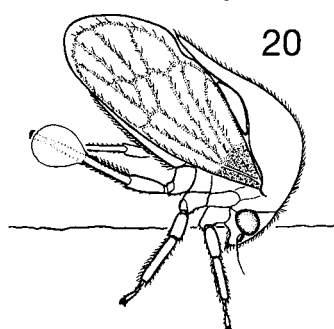
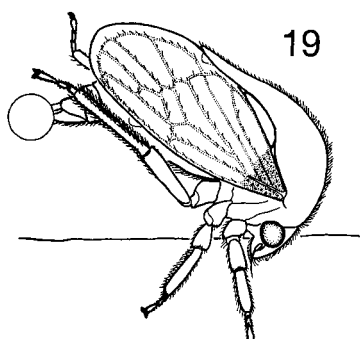
Nymph

In two 4th instar and one 5th instar nymphs anointing was recorded some 120–160 min after moulting. It was found to be identical to the bathing of the *Oncopsis* nymphs.

SECRETION. The only slightly opalescent white fluid leaves no visible sediment. It contains peculiar irregularly dimpled spindle-like particles of 4–10 μm in length and 0.4–1.5 μm in width (Fig. 25). The same particles were found on the cuticle and in the crushed Malpighian tubules of *Macropsis vicina* (Horvath).

Adult

Anointing was observed in two individuals 150–180 min after moulting. The anointing is similar to the second step of that in adult *Cicadella*: 2–4 secretory drops are transferred onto the tegmina and spread over them by the hind tibiae. Then the crossed tibiae push the liquid forward to the scutellum and pronotum where the fore legs pick it up and spread it



Figs 19–21: *Gargara genistae*, adult. Anointing.

SECRETION. The fluid is only slightly white opalescent, and leaves no visible sediment. No particles were found in it after examination by SEM.

Adult

Anointing was recorded in two wingless individuals 260 and 360 min after moulting. Two steps involved in the process are as follows:

1) The insect raises its body, taking nearly vertical position, balancing itself on its rostrum and face alone. Then the tip of the abdomen is curved downwards and a single secretory drop flows from the rear end. Both hind legs pick it up and transfer it onto the base of the abdomen, close to the hind coxae. Now all the legs begin to move slowly distributing the fluid between their bases over the ventral surface of the thorax. Thereafter the insect rests.

2) Roughly 20 min later the insect raises its body again and secretes in succession 4–6 droplets, picking them up alternately by its hind tibiae which transfer each droplet to the

onto the face. After the secretion dries the insect grooms itself violently.

SECRETION. The white opaque, opalescent liquid leaves a white sediment consisting of BS.

Subfamily Agalliinae

Agallia venosa (Fourcroy, 1785)

Nymph

Bathing was observed in one 5th instar nymph 5.5 h after moulting on the wall of the tube. A single drop of fluid was excreted onto the glass. Then the insect dipped its head into the secretion, laid down and overturned onto its back. No grooming was recorded thereafter.

SECRETION. The fluid was almost transparent, it left no visible sediment on the glass. No particles were found in this sediment as well as on the nymph cuticle after examination by SEM.

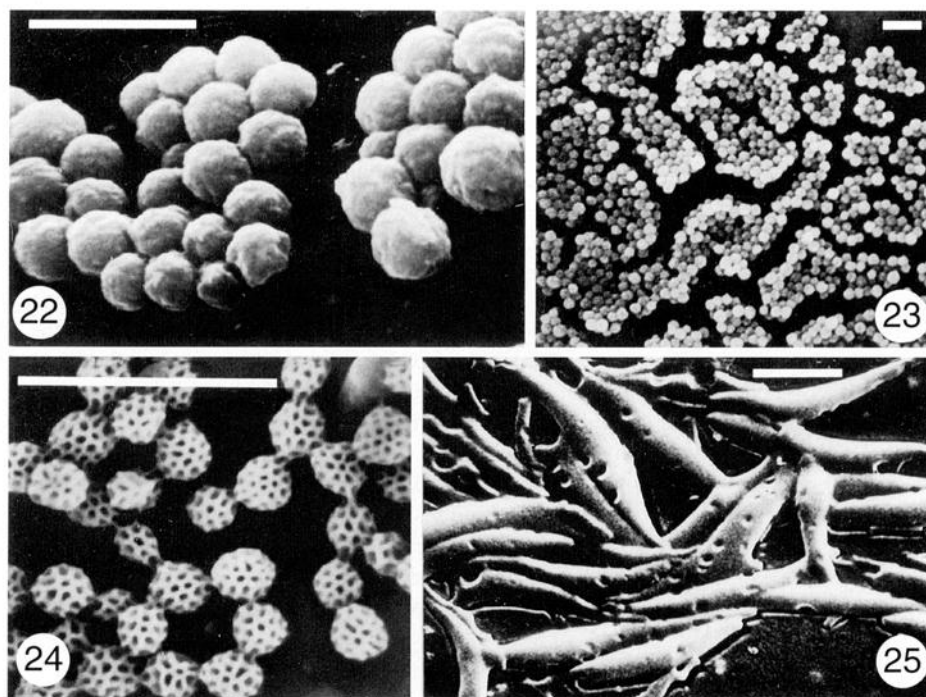
NOTE. Adults are well-covered with BS.

Subfamily Ulopinae

Ulopa reticulata (F., 1794)

Nymph

Bathing, similar to that of *Agallia* nymph, was recorded in two 5th instar nymphs roughly 8 and 12 h after moulting. After the drop of fluid is secreted onto the heather leaflet the nymph turns around and dips its head into the fluid, then lays down into the drop on its side. No grooming was observed.



Figs 22–25: Particles secreted in anointing. 22 – spheroids on the cuticle of the 5th instar nymph of *Oncopsis flavicollis*; 23 – the same in the sediment of fluid excreted by the 4th instar *O. flavicollis* nymph while anointing; 24 – brochosomes on the hindwing of adult *O. flavicollis*; 25 – spindle-like particles in the sediment of the fluid excreted by the 5th instar nymph of *Macropsis graminea* while anointing. Scale bar: 3 μ m (all Figs).

lateral side of the 9th abdominal tergum. Two secretory drops are situated on the sides of the pygofer. Thereupon the leafhopper presses the abdomen to the underside of the tegmina and by circular movements of its tip spreads the fluid. As a result, at least the apical parts of tegmina, ventrally, and the apical part of the abdomen, dorsally, become moistened. Then the insect adopts its normal posture. No grooming was recorded thereafter.

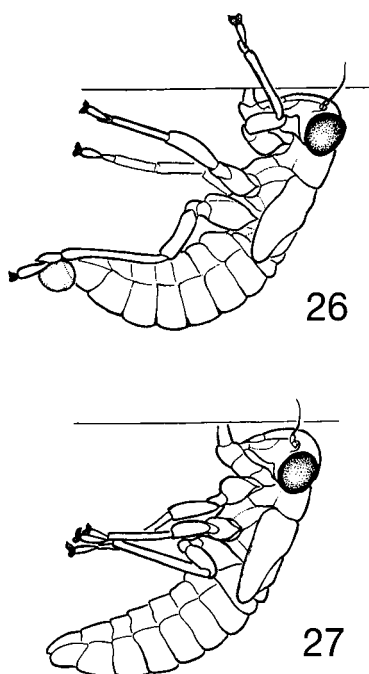
SECRETION. The fluid is white opaque, opalescent, and leaves a white sediment consisting of BS.

Family Membracidae
Subfamily Centrotinae
Gargara genistae (F., 1775)

Nymph

Anointing was observed in 7 nymphs of 2nd–5th instars 150–260 min after moult. It was found to be considerably similar to the bathing of *Macropsinae* nymphs.

The nymph, resting with its stylets embedded in the host-plant, suddenly starts to crawl. A few seconds later it stops, raises the abdomen and secretes a single drop of fluid onto



Figs. 26–27: *Populicerus nitidissimus*, 5th instar nymph. Washing of legs.

the dorsal side of the pygofer (Fig. 14). The next moment the drop is shifted by the hind legs to the ventral side of the abdomen and the body is pressed to the plant surface (Fig. 15). As a result the fluid is smeared onto the whole underside of the body. Later the nymph dips its head repeatedly into the remains of the drop, lays down on its side and turns onto its back (not clinging to the plant, even on the underside), similar to nymphs of *Macropsinae* (Figs 16–18). As the nymph does so, its pygofer is bent upward and pressed to the dorsum. The whole cuticle of the insect becomes moistened. The act is finished in a characteristic way: the nymph crawls on the plant rhythmically raising its abdomen and leaving a moist trail behind it. Finally it rests, dries and returns to feeding. No grooming was recorded.

SECRETION. The fluid is only slightly white and opalescent, leaves no visible sediment. No particles were found in samples of secretion and on the cuticle of nymphs after examination by SEM.

Adult

Anointing was observed in 4 adults about 180 min after moulting. In one individual the second act of anointing was recorded several hours after the first.

The caudal part of the body is raised, and both hind legs pick up a single drop secreted from the anal tube (Fig. 19), knead it a little under the abdomen (Fig. 20) and then they smear the fluid onto the tegmina (Fig. 21). Later the insect rubs its mid-legs and the underside of the abdomen with moist hind legs, and thereupon rests. Anointing is followed by a few grooming movements.

SECRETION. The fluid is white, opalescent, semitransparent, leaves no visible sediment. No particles were found after examination by SEM in the samples of fluid as well as on the cuticle of the treehoppers prepared immediately after anointing.

WASHING OF LEGS

This distinctive type of behaviour, unassociated with moulting, was observed in leafhoppers of different taxa and stage, i.e., in nymphs and adults of *Cicadella viridis*, adults of *Macropsis graminea*, nymphs of *Populicerus nitidissimus*, *Idiocerus stigmatalis* and *Graphocraerus ventralis*.

The insect picks up a single drop of transparent fluid from the apex of the abdomen with its hind legs and distributes most of it over the tarsi by kneading movements of all the six legs (Figs 26, 27). When doing so on the upper side of the plant, the insect balances itself on its rostrum and face. Whereas, on the underside, it is suspended on its rostrum alone.

Finally, the insect adopts its normal posture, standing with its wet legs on the plant surface. No particles were found after examination by SEM in samples of fluid emitted by a 4th instar nymph of *P. nitidissimus*.

DISCUSSION

ANOINTING BEHAVIOUR

General pattern

As noted by Navone (1987), anointing occurs after moulting. The present data indicate that the first act occurs usually 1–3 hours after moulting, depending on species and stage. Subsequent acts were observed in adults of *Vilbasteana*, *Balclutha* and *Gargara*. In all probability anointing recurs several times after the final moult, gradually decreasing in frequency.

Anointing and grooming

Table 1 shows a strong correlation between grooming behaviour and secretion of BS ($P = 0.002 < 0.05$, Fisher's exact probability test). In most leafhoppers studied (excl. *Ulopa*) anointing with fluids containing BS was followed by violent grooming, whereas no or only a few grooming movements were observed after anointing with fluids without BS. Acts of grooming never occur between the moult and the first act of anointing. Grooming behaviour of leafhoppers has a special function. In this process the dry mass of BS is distributed over the entire surface of the cuticle, forming a more or less regular covering. This behaviour, basically similar in adult leafhoppers of different subfamilies, was described in detail for adults of *Rhytidodus*, *Zyginidia* (Navone, 1987), and *Vilbasteana* (Rakitov, 1992). Compared with anointing, the series of grooming movements occur more often. In laboratory experiments, for example, the adults of *V. oculata* spent in total 1.5–3 minutes grooming every hour. Quite often several leafhoppers reared together groom simultaneously. As a whole, the grooming behaviour appears to be associated primarily with some external stimuli, but not with a contamination of the cuticle. It is possible that the repetitive grooming of the cuticle serves to maintain some optimal conditions of the brochosomal covering, such as its smoothness, regularity and continuity.

Acts of anointing

As was found in *Zyginidia* (Navone, 1987), it is probable that acts of anointing are the same in all five nymphal instars. In terms of behavioural patterns, two types of nymphal anointing may be distinguished (Table 1). Nymphs of *Cicadella*, *Vilbasteana*, *Cicadula*, *Graphocraerus* and *Iassus* exhibit a two-step process, in which the secretion is applied immediately to the body surface: at first on the legs and ventral side of the thorax, and shortly afterwards on the dorsal body side. These acts were found to be very similar in different species, and they are referred to as 2-step smearing in Table 1. Similar behaviour was reported for the 1st and 5th instars of *Zyginidia pullula* (Boheman) (Navone, 1987).

By contrast, the nymphs of *Oncopsis*, *Macropsis*, *Agallia*, *Ulopa* and *Gargara* bathe in the secretory drops on the plant surface. In the *Gargara* such a bathing differs somewhat from the behaviour of leafhopper nymphs (initial posture, involvement of hind legs, final path) but, for the most part, resembles it strikingly.

Bathing is associated clearly with the structure of nymphs. Those of Macropsinae, Ulopinae and Membracidae are similar in having relatively short hind legs and a dorsomedially raised abdomen, often in the form of a crest. The short hind legs do not appear to be suited well to anointing and grooming, whereas the shape of the abdomen does not permit it to curve upwards and forwards, as other nymphs do in anointing (smearing). Nymphs of *Agallia venosa* resemble more typical cicadellid nymphs in the structure of their hind legs and abdomen, but, in some other species of Agalliinae, a clear abdominal crest is developed. It is also notable that nymphs of Macropsinae are unable to leap, like those of the Membracidae and in contrast with, probably, all other leafhopper nymphs.

The absence of nymphal anointing in *Rhytidodus* (Navone, 1987), as well as in *Populicerus* and *Idiocerus*, suggests that it may be typical of the subfamily Idiocerinae. The presence of a copious waxy covering on the body of the *Populicerus nitidissimus* nymphs appears to be associated with this.

All the acts of imaginal anointing described herein may roughly be classified as smearing, but, in detail, they vary considerably between species and, moreover, in successive acts of the same individual. *Cicadella* and *Balclutha* (in the first act of anointing) exhibit a two-step process, smearing the first portion of secretory fluid onto the legs and ventral side of the thorax, as in their nymphs. In other species (excl. *Ulopa*) the adults transfer the fluid to the tegmina immediately.

Two strategies in the distribution of the secretion may be recognized. Adults of *Populicerus*, *Oncopsis*, *Macropsis*, *Cicadella* and *Balclutha* (in the first act of anointing) spread the fluid over the tegmina, scutellum, pronotum and head. The same was found in *Rhytidodus* (Navone, 1987). After the secretion hardens, it is distributed further by acts of grooming as a dry matter. By contrast, in *Vilbasteana* and *Zyginidia* (Navone, 1987), as well as in *Balclutha* (in the second act of anointing), the fluid transferred onto the tegmina is allowed to dry here as wax-area spots. Thus the secretion, composed of BS, is distributed over the cuticle in the process of grooming alone. A special rugose area near the costal margin of the tegmen in the Typhlocybiinae, where the secretory droplets are placed (Navone, 1987), provides an attachment for the mass of BS to the tegmen. Adults of *Balclutha*, in the first act of anointing, spread the fluid over the tegmina, and it is probable that this promotes better adhesion of the brochosomal spots formed in the second. The difference between the first and second acts of anointing in *Alebra* and *Eurhadina* may be explained in the same way. Storey & Nichols (1937) reported that *Cicadulina mbila* transfers 2–3 droplets onto the tegmina, where they dry as white spots. The authors did not observe the insect immediately after moulting, and it is possible that they described only the latter variant of anointing.

Ulopa exhibits the most peculiar imaginal anointing process, which may be considered as a very special two-step smearing. The hind legs transfer the fluid onto the body but do not spread it. Instead, the fluid is distributed by the movements of the coxae and abdomen, and anointing is not coupled with grooming. As a result only the ventral parts of the thorax, the dorsal side of the abdomen and the inner surface of the tegmina are covered with BS. These peculiarities appear to be partly associated with the coarsely pitted and well sclerotized surface of the tegmina, scutellum, pronotum and head. Moreover, the absence of grooming appears to be related to the characteristic absence of the rows of spines on the hind legs in *Ulopa*.

Imaginal anointing in *Gargara* differs from the smearing of the leafhopper adults, primarily, in that a single drop only is excreted. It is picked up and kneaded with both the hind legs, – thus resembling the first step of the two-step anointing in leafhoppers. However, the fluid is not transferred onto the mid- and fore legs and ventral parts of the thorax, but smeared directly onto the tegmina. In this act only the tegmina and the distal parts of the hind legs are covered thoroughly with the secretion. It is evident that irregular grooming movements (associated with both the absence of BS in the fluid and the absence of the rows of spines on the hind legs) cannot distribute any secretory material over the body surface. Hence, the whole process appears reduced or even atavistic in comparison with the normal act of anointing in leafhoppers.

No anointing behaviour was recorded in adults of *Iassus lanio*. This appears to be related to the presence of the waxy secretion on the integument in this species (Rakitov, 1995). The absence of anointing in *Iassus* is not general for the Iassinae, because the brochosomal covering is found on the cuticle in *Batracomorphus* and in other members of this subfamily.

Secretion

It had been suggested that the opaque white secretion smeared onto the cuticle by leafhoppers invariably contains BS. New evidence indicates that this is not so for nymphs of several subfamilies of leafhoppers and for both nymphs and adults of *G. genistae* (Table 1). The composition of the BS-free fluids they secrete is obscure. Fluids secreted by nymphs of the Macropsinae contain peculiar particles originated in the Malpighian tubules (Figs 22, 25), whereas no particles (of sufficient size to be recognized after examination by SEM) were found in secretions of nymphs in *Iassus*, *Vilbasteana*, *Agallia*, *Ulopa* and both nymphs and adults of *Gargara*. However, all these BS-free secretions appear distinct from faeces, being more or less opaque, white and opalescent. This indicates that such BS-free fluids contain unknown secretory material of a colloidal nature. Ultrastructural studies (in progress) suggest that most probably this secretion also originates in the Malpighian tubules, which produce various materials in addition to BS. The presence of another secretory component besides BS in the BS-containing fluids cannot be excluded.

As can be seen from the figures, the volume of fluid excreted in anointing by nymphs is very large in comparison with their body size. This suggests that, in such cases, the products of the Malpighian tubules should be considerably diluted by the contents of the gut.

Relation to the Cercopoidea

Among the non-membracoid Auchenorrhyncha, the secretory activity of the Malpighian tubules is known only in the froth-producing and tube-building nymphs of the superfamily Cercopoidea. The highly specialized Malpighian tubules of these nymphs produce large amounts of mucopolysaccharides and proteins, in granular or fibrillar form, which, probably, play an important role in the formation of froth and in the building of calcareous tubes (Strümpel, 1983). Dietrich & Deitz (1993) regard Cercopoidea and Membracoidea as sister groups. More histochemical and ultrastructural data are needed to prove that secretory specializations of the Malpighian tubules are homologous in both superfamilies. If this is so, it is possible that the external use of secretory products of specialized Malpighian tubules may already have evolved in a common ancestor of the Cercopoidea and the Membracoidea. Further there is some evidence that nymphs of Cicadoidea, usually placed close

placed close to the Cercopoidea, can daub the walls of their burrows with fluid excretions, and it is suggested that the latter also contain special products of the Malpighian tubules (Boulard, 1965).

Function

The function of anointing in leafhoppers and treehoppers is enigmatic. In leafhoppers which secrete BS, this behaviour results in the formation of brochosomal covering on the cuticle. It is suggested that this serves as a form of protection like the waxy covering of aphids, whiteflies and some other small insects. Its functions may be water repellence, extra waterproofing, anti-molestation, anti-contamination, anti-fungal protection or thermoregulation (Günthart, 1977; Arzone, 1986; Navone, 1987). A general absence of waxy coverings in the Cicadellidae, in contrast with other small Auchenorrhyncha, is in accord with this interpretation (Rakitov, 1995), but, as for waxy coverings in other insects, these suggestions have yet to be proven experimentally. Alternatively, Day (1993) suggested that BS may serve as vectors of aggregative pheromones, but there is no evidence of aggregative behaviour for the majority of leafhoppers. The regular brochosomal covering prevents the adhesion of water or faeces to the cuticle. Undoubtedly such a property is of great importance for small insects, especially those excreting honeydew. In many leafhopper species such a role for the covering appears to be undeniable. However, it is difficult to imagine that it may be the sole and primary function of BS in the family Cicadellidae. BS-free anointing in leafhopper nymphs and treehoppers suggests that the anointing, as a whole, cannot be explained in this way. It is not known whether the BS-free secretions modify any properties of the integument. Nymphs of the Macropsinae, after anointing, remain capable of being moistened. Unfortunately, very little is known about the chemical composition and properties of the secreted matter, and it is difficult to explain this phenomenon with only general principles and analogies. Removal of waste products excreted by the Malpighian tubules and the activity of these organs are associated with moulting in many insects. It is evident that this is linked with the origin of the anointing behaviour, but not with its present function.

Leafhoppers and treehoppers

A recent revision of the superfamily Membracoidea (Deitz & Dietrich, 1993) considered it to be composed of four families: the Cicadellidae, the Membracidae and two smaller families, the Aetalionidae and the Melizoderidae. The authors noted that BS were found on the cuticle in the Cicadellidae only. Indeed, there is no conclusive evidence on the production of BS in any non-cicadellid Membracoidea. The Malpighian tubules of the Membracidae are very similar in shape to those of the Cicadellidae, both having an inflated secretory region in the middle, but no detailed ultrastructural investigation of these organs in treehoppers has been made. Gouranton & Maillet (1967) state that they found BS in the Malpighian tubules of *Centrotus cornutus* (L.), but they provided no further details or photographs. The BS were reported for some Australian treehoppers by Day (1993) but, as in the previous example, no details were given. At the same time no BS were found on abdominal integument in treehoppers of various groups by comparative SEM investigation (Dietrich, 1989). BS were not found on the cuticle nor in the Malpighian tubules of *Centrotus cornutus* (L.) and *Gargara genistae* (F.) (pers. observ.) as well as in the fluid excreted during anointing by the latter. Therefore, pending further studies, it is possible

that the above records of BS in the Membracidae are erroneous and the only group producing BS is the Cicadellidae. However both families share anointing behaviour.

There is no generally accepted opinion on the phylogenetic relationships between families and subfamilies of the Membracoidea. Dietrich & Deitz (1993) consider the Cicadellidae and the Membracidae as sister groups, with the production of BS as a putative synapomorphy of the Cicadellidae. If this is correct: 1) treehoppers exhibit an ancestral type of anointing; 2) the nymphal bathing shared by the Membracidae, Macropsinae, Agalliinae and Ulopinae indicates the relative primitivity of the subfamilies; 3) in the leafhopper lineage BS were excreted initially at the adult stage only, which implies a process of imaginalisation of nymphs (in structure and behaviour) in more derived leafhopper subfamilies (e.g., in Cicadellinae and Deltocephalinae). Alternatively, Hamilton (1983) regards the Membracidae and the Aetalionidae as derived from the Cicadellidae, and this opinion agrees with paleontological data (Shcherbakov, 1992). This suggests that the Membracidae have lost the production of BS secondarily. The ancestral or derived nature of nymphal BS-free anointing remains obscure in this case. Bathing of nymphs may be an ancestral condition retained in the Membracidae, Macropsinae, Agalliinae and Ulopinae or, alternatively, unless not developed independently, it is a synapomorphy of these taxa. The last assumption is supported by some morphological characters, mentioned by Evans (1971), who pointed out the affinity of the Macropsinae with the Ulopinae and Agalliinae (also in the structure of their nymphs) and with the non-cicadellid *Melizoderes*, and Hamilton (1983), who placed the Ulopinae close to the Membracidae and Aetalionidae. There is no doubt that further extensive studies on the production of BS and anointing could contribute much to the clarification of phylogeny of the Membracoidea.

WASHING OF LEGS

Generally resembling the first part of the two-step act of anointing, the washing of legs should be regarded as a separate type of behaviour, being distinguished from anointing by the following features: 1) it is not associated with moulting, recurring irregularly throughout the insect's lifetime; 2) it is not coupled with subsequent smearing of the fluid onto the dorsum; 3) normally, only the distal parts of the legs are covered with the secretion and 4) the latter appears to be transparent.

In contrast with anointing, these curious rituals appear to be associated with external stimuli. Quite often one might observe simultaneous washing in several leafhoppers. In one individual the acts of washing were observed more than four times in succession. It is probable that leafhoppers use their faeces in washing. Both the transparency of the secreted fluid and the ability of leafhoppers to repeat these manipulations many times in succession support this conjecture. Behaviour of the same kind was described as "another type of collecting and distributing brochosomes" by Navone (1987) in adult *Rhytidodus decimusquartus*, after moulting and a typical anointing act. Only the legs were moistened in this example with fluid, and the excretory droplet shown on the photograph is transparent in comparison with the droplets secreted in anointing. The "second method of defaecation" described by Storey & Nichols (1937) in the nymphs of *Cicadulina mbila* also more likely resembles washing of the legs than true anointing.

The role of the washing of the legs is obscure: it may be cleaning of tarsi, removal of pathogenic spores, better contact with plant surface, etc. In terms of its origin this behaviour seems to be a by-product of anointing.

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