

Mimicry and ultrastructural analogy between the semi-aquatic grasshopper *Paulinia acuminata* (Orthoptera: Pauliniidae) and its foodplant, the water-fern *Salvinia auriculata* (Filicatae: Salviniaceae)

by

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(Accepted for publication: July, 1993).

Abstract

The semi-aquatic grasshopper *Paulinia acuminata* is mimetically protected by a striking similarity to its foodplant, the floating fern *Salvinia auriculata*. A SEM study of the cuticula of young grasshopper nymphs and the *Salvinia* leaf surface reveals a similar ultrastructure of epicuticular waxes which makes both organisms extremely water repellent.

Keywords: *Salvinia*, *Paulinia acuminata*, mimicry, epicuticular wax, ultrastructure, Neotropics.

Introduction

Plants and their predators show a complex pattern of evolution and co-evolution in all aspects including their surfaces (survey in JUNIPER & SOUTHWOOD 1986). The present paper describes mimetism and a striking convergence on the microsculptural level between the semi-aquatic grasshopper *Paulinia acuminata* (DE GEER, 1773) and its host, the water-fern *Salvinia auriculata* AUBLET, 1775.

Salvinia auriculata, a member of the highly specialized water-fern family Salviniaceae (survey in SCHNELLER 1990), is very common in nutrient-rich water throughout the Neotropics; it also occurs in West Africa. The plant is completely rootless, the horizontal stem floating just below the water surface bears whirls of three leaves (survey in CROXDALE 1978, 1979). The solitary ventral leaf is deeply submerged, strongly divided and overtakes the function of roots. The floating two dorsal leaves are entire, simple and visible on the water surface (Figs. 1, 2). The floating leaves are covered by dense multicellular hairs and epicuticular waxes which cause an extreme water repellency (Figs. 3, 4).

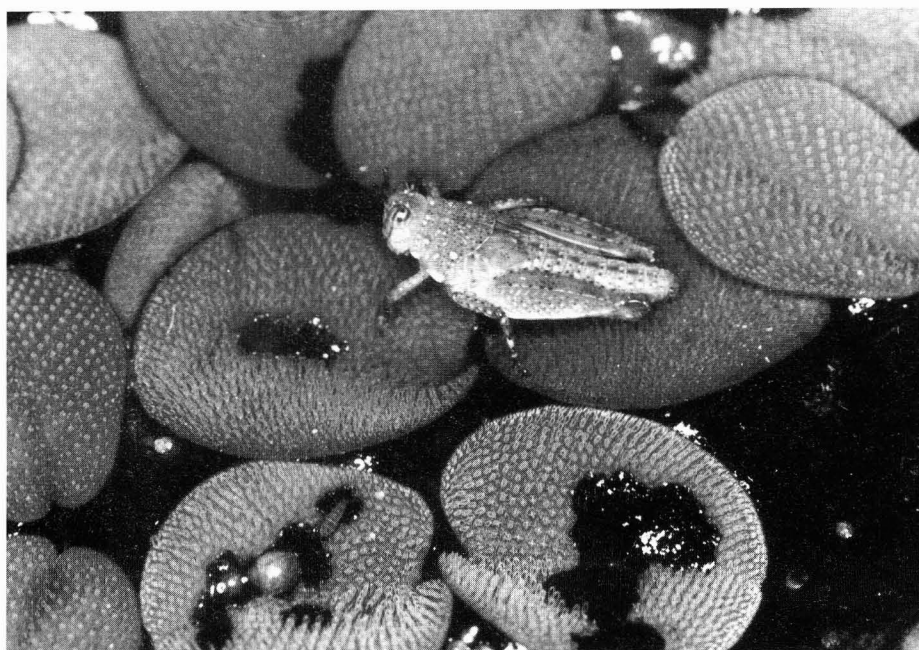


Fig. 1:

Younger nymphs of *Paulinia* feeding on leaves of *Salvinia* (food marks in the foreground). The slow moving nymphs mimic the colour and surface texture of the leaves; both are unwettable.

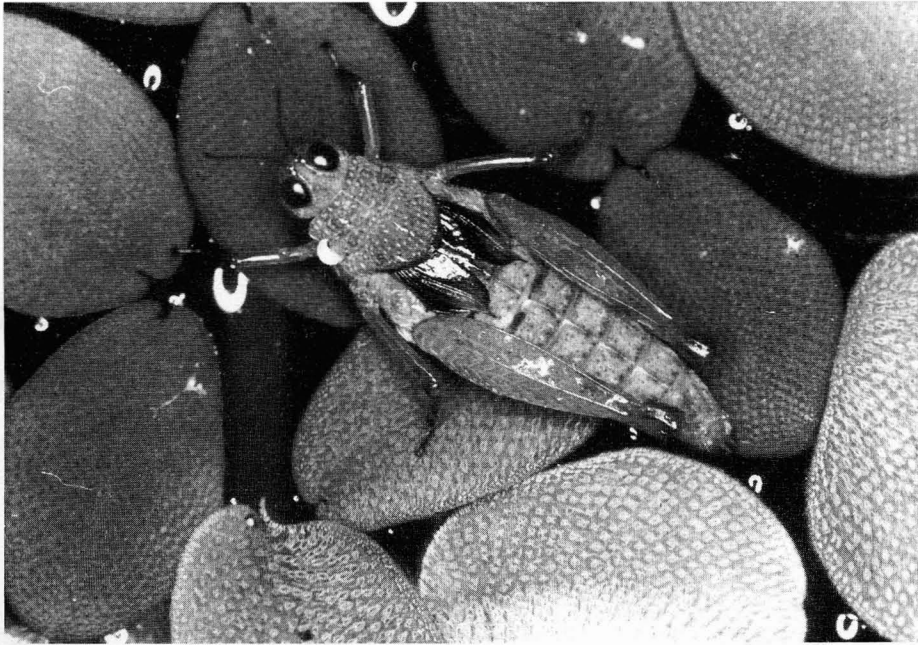


Fig. 2:

Older nymphs and adults of *Paulinia* are larger than *Salvinia* leaves and develop a somatolytic pattern: the animal is optically divided into two portions by the dark wink rudiments mimicing two *Salvinia* leaves with the dark water surface between the leaves.

A considerable number of South American grasshoppers inhabit aquatic biota. Some terrestrial forms live on the shore, but occasionally enter the water to feed or oviposit on floating plants. They belong to the subfamily Leptysminae (Acrididae), while the semi-aquatic ones form a separate family, the Pauliniidae, which contains three genera, the monotypic *Paulinia* (BLANCHARD, 1845) and *Waehneriella* (GÜNTHER, 1940), and the genus *Marellia* (UVAROV, 1929). The latter contains four similar species which could well represent intraspecific variations of a single, widespread species found from Argentina to Central America and Trinidad (CARBONELL 1981). The most suspicious adaptation of these "aquaticoles" (UVAROV 1977) are broadened hind-tibia which serve for swimming and diving. These oar-shaped legs were name-giving in *Marellia remipes* UVAROV, 1929.

The systematic position of the clearly aberrant but artificial family Pauliniidae is unclear. A general depressed shape of the body, eyes on the upper part of the head and reduced pretarsal arolia are features of ground-living forms. This can be interpreted either as a case of convergence due to living on the horizontal surface of floating leaves, or as a phylogenetic relation to the ancient family of mainly ground-living Ommexechidae (CARBONELL 1957).

P. acuminata is a variable species with long- and short-winged forms which is found in most of subtropical and tropical South America, from Uruguay and the province Buenos Aires (Argentina) to Central America and the island of Trinidad (CARBONELL 1981).

Material and methods

Plants of *Salvinia auriculata* had been collected for cultivation in the Botanical Garden of the University of Bonn (FRG) by M. Koenen (Bonn) in October 1990 in a small ephemeral water pond on the coastal plains in North West French Guyana close to the border of Surinam. In cultivation the plants showed feeding marks; closer investigation revealed many nymphs of a grasshopper which were almost invisible due to their perfect camouflage of the similarly colored leaves of *S. auriculata*. Nymphal stages and later adult specimens of *P. acuminata* were caught and killed in a freezer: no organic agents were used for killing or preservation because of the delicate wax cover of *Paulinia*.

Paulinia and fresh leaves of *Salvinia* were air-dried and prepared by cold-coating for examination in a scanning electron microscope (Cambridge S 200). The general micromorphology of the *Salvinia* surface was studied in leaves dehydrated by the critical-point-method. As this method destroys the ultrastructure of epicuticular waxes, the latter aspect was investigated in air-dried material (survey of SEM preparation method for waxy surfaces in BARTHLOTT & WOLLENWEBER 1981).

Results

Mimetism and biology of *P. acuminata*

While the younger nymphal stages of *P. acuminata* are smaller than leaves of *Salvinia* and possess the same green coloration (Fig. 1), later stages are clearly larger than the 0.5 cm long and 0.3 cm broad leaves (Fig. 2). However, even the older animals are difficult to detect because they now possess dark wing rudiments which can be confused with gaps between the *Salvinia* leaves (Fig. 2). This somatolytic effect is strengthened by an extraordinary slow movement of the grasshoppers. *P. acuminata* is sedentary and stays on its food plant for long periods of time. Especially nymphs jump off only after strong irritation and evidently rely on their mimetic coloration as an efficient anti-predator defence which explains the accidental collection of nymphs with plant material. The only conspicuous feature of adult individuals are white antennal tips which probably facilitate intraspecific optical communication, as is known of other dumb species (RIEDE 1987).

Adults jump only at higher temperatures. On overcast days or with lower temperatures they dive away by walking backwards rapidly and entering into the water abdomen-first. Submergence is an effective means of temperature regulation in subtropical regions, and CARBONELL (1964) reports that on cooler days only the head is looking out of the water, which is often warmer than the air.

Micromorphology of *S. auriculata*

The upper side of *Salvinia* leaves shows several rows of trichomes appearing like small crowns in the stereo microscope. The multicellular hairs of *Salvinia auriculata* are characteristically arranged in groups of four (Fig. 3). The extreme water-repellency of the leaves is highly remarkable. Intentionally subdivided plants reach immediately the water surface by the air enclosed in the intercellular space and under the crown hairs.

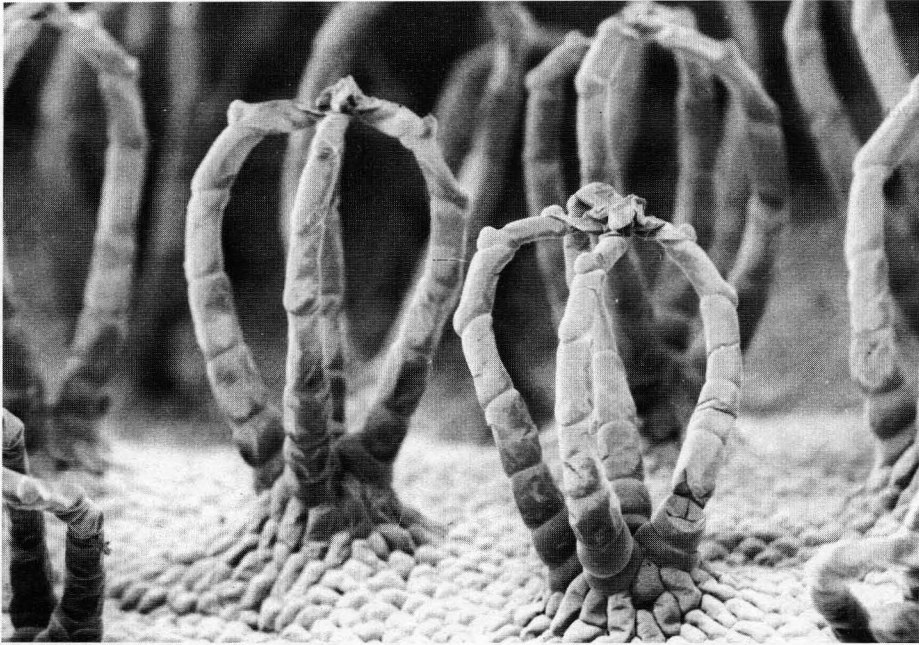


Fig. 3:
Salvinia, at low magnification the crown-like multicellular hairs on the leaves. Higher magnification of single cells (see Fig. 4) reveals a cover of epicuticular waxes. 1:10.

An additional barrier at the surface, responsible for the high water-repellency, can be seen only at higher magnifications in SEM (Fig. 4). The waxes of *Salvinia* are composed of extremely thin rodlets perpendicular to the surface. They are $0.5\ \mu\text{m}$ high and broaden slightly towards the base. Their mean distance is approximately $0.5\ \mu\text{m}$. Between the rodlets extraordinarily thin wax filaments, difficult to visualize even with high resolution SEM, having a diameter of $0.03\ \mu\text{m}$ can be found. These crystalloid waxes occur on the leaf cells as well as on the trichomes.

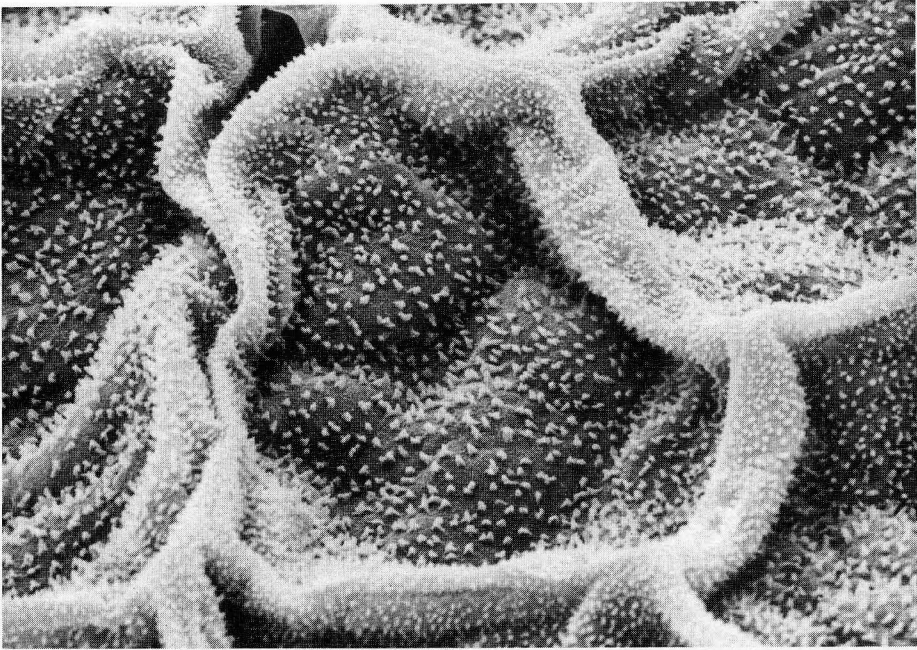


Fig. 4:
Salvinia, single epidermal cell with dense cover of epicuticular wax. SEM 1:2200.

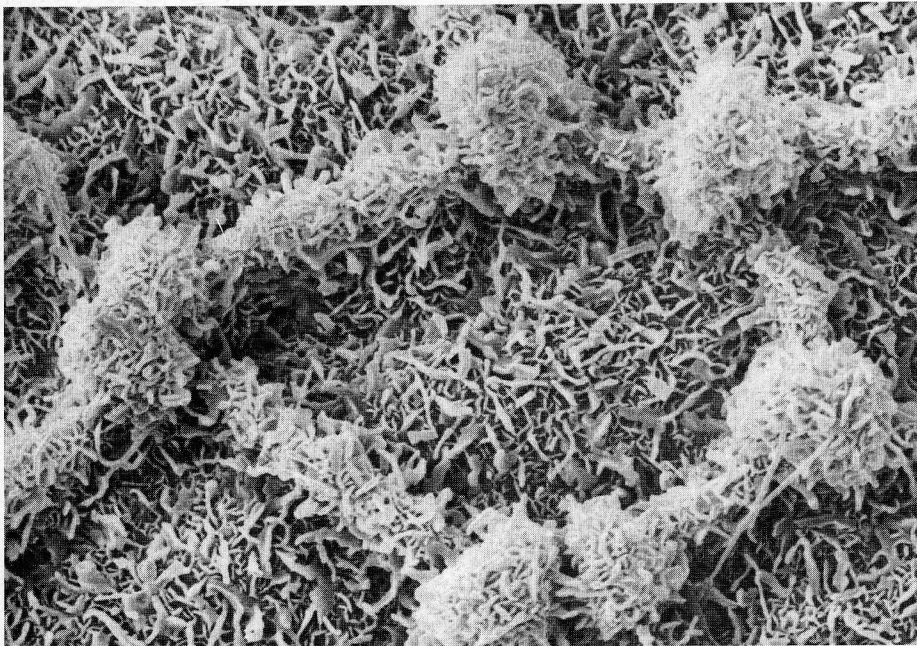


Fig. 5:
Paulinia, cuticle of young nymph. The cuticle is always covered by epicuticular wax, arranged in pentagons of similar size as in *Salvinia* (Fig. 4), thus causing a striking functional analogy. SEM 1:2200.

Micromorphology of the cuticula of *P. acuminata*

Young nymphs of *Paulinia acuminata* are like *Salvinia* leaves highly water-repellent. The whole body is covered by waxes; only tarsae, eyes, antennae and cerci are free from these waxes which allows good contact to the floating leaves and unimpaired sensory perception (Fig. 6). Higher magnification reveals slightly elongated, hexagonal cells with a diameter between 10 and 20 μm , showing a margin of coherent \pm smooth walls of 1 μm height and tapering into single tips (Fig. 5). A higher magnification shows that the waxes of *P. acuminata* are composed of irregularly arranged smooth platelets about 2 μm long, 0.15 μm broad and a height of nearly 0.5 μm .

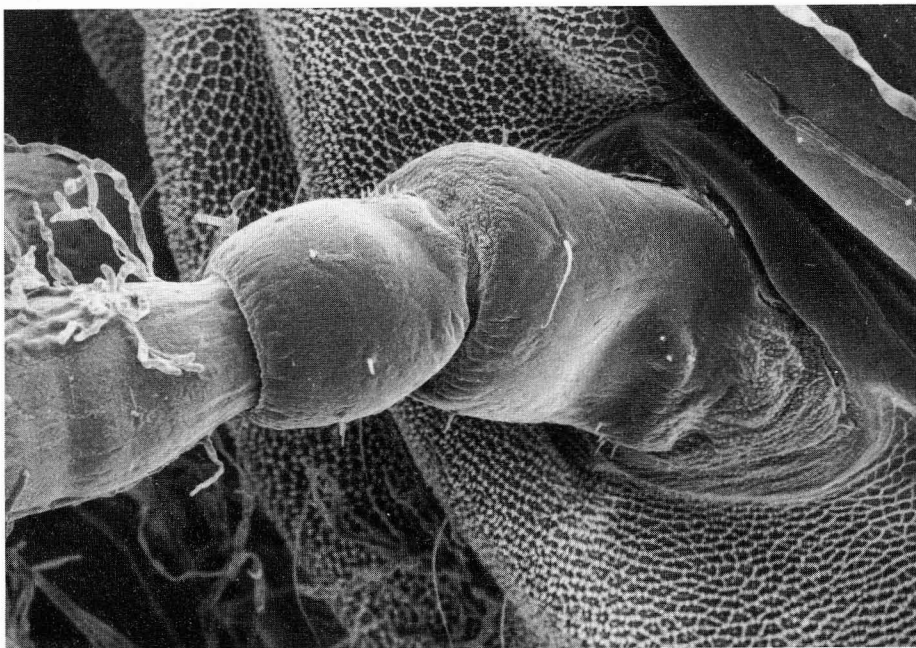


Fig. 6:
Paulinia, young nymph. Basal portion of the antenna. The fine reticulate pattern in the background is caused by the waxy cells pictured in figure 5. Microsculpturing and waxes are absent on the antennal surface. SEM 1:150.

At a later nymphal stage, when the grasshoppers are larger than the *Salvinia* leaves, the dark wing rudiments occur. The animals are now well wettable and the above described waxes are absent (Fig. 7).

Treatment with warm xylene removes the waxes from *Salvinia* leaves. Similar treatment also removes the waxes from *Paulinia*, but the above described tips of the cells can then be seen clearly. A higher number of very small pores, tentatively the location of wax extrusion, are found in the central portion of the outer cell walls. Such pores do never occur in plants, where the process of wax extrusion is practically unknown even nowadays (survey in BARTHLOTT 1990).

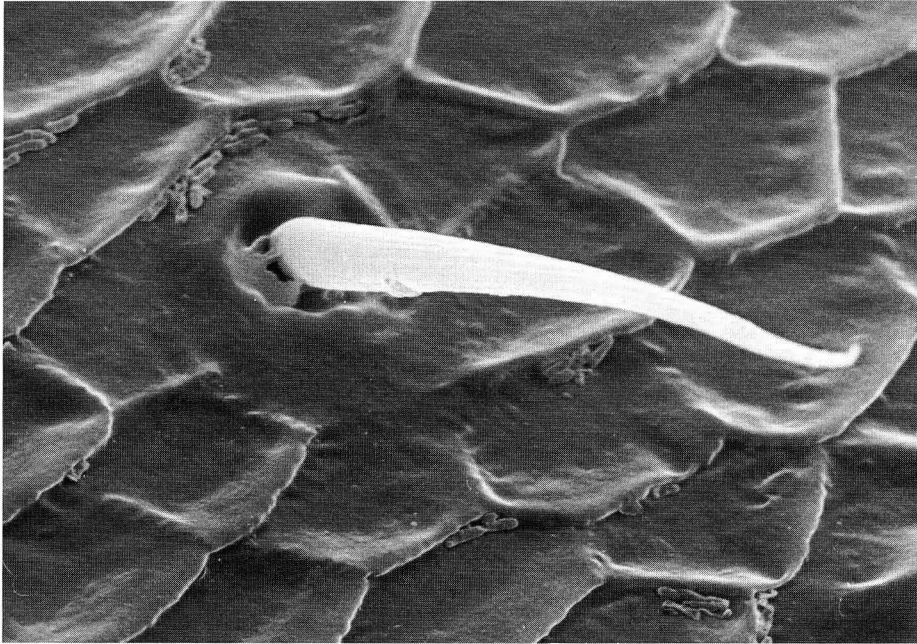


Fig. 7:

Paulinia, old nymph. The surface of the abdomen is smooth and without epicuticular waxes. The animal is wettable. SEM 1:1000.

Discussion

Mechanisms of water-repellency and function of waxes

The combination of multicellular crown hairs and epicuticular waxes results in the extreme water-repellency of *Salvinia* leaves so that even in a strong storm the plants do not capsize or sink. Other floating plants in the same environment, e.g. *Pistia stratiotes* (Araceae) or *Phyllanthus fluitans* (Euphorbiaceae), are also highly water-repelling by the same mechanism. High resolution SEM reveals a striking similarity in the dimensions of surface roughness in *Salvinia* and *Paulinia* (Figs. 4, 5). The size of cells ranges from 10 to 20 μm and the wax level from 1 to 2 μm which is characteristic for many hydrofuge plant surfaces. The high water-repellency can be achieved only by a combination of hydrophobic waxes and an additional surface roughness (HOLLOWAY 1970). The investigated plant surfaces as well as the animal fulfil these conditions.

It seems that waxes on insects appear rarely and are developed only under specific conditions. They are mostly constructed by composed rodlets similar to the so-called *Strelitzia* waxes from plants (FRÖLICH & BARTHLOTT 1988). Similar extrusions have been shown by BAKER & JEFFREE (1981) and SARGENT (1988) from the "white fly", an insect commonly found in green houses, and on cicada nymphs by MESSNER & ADIS (1992).

Function of water-repellency in grasshopper nymphs

The main function of epicuticular waxes of insects is thought to be the reduction of transpiration (HADLEY 1986; HADLEY & HENDRIKS 1985) which, however, seems not necessary for animals which have no difficulty with water-supply. Paradoxically, both xeric and aquatic environments require development of waxes which may be explained by the necessity of repelling water coming from the inside or the outside, respectively. Thus, the similarity of ground-living "terricoles" and "aquaticoles" (UVAROV 1977) could extend to the ultrastructural level: protection from desiccation is also an efficient protection against getting wet and may have been the necessary preadaptation for the conquest of the aquatic habitat by *Paulinia*. Therefore, it would be interesting to search for similar waxes in other semi-aquatic grasshoppers, as well as in terrestrial Ommexechidae.

Contrary to *Salvinia* the grasshopper changes its wettability through its life cycle. The change from a waxy, water-repellent cuticle in nymphs to a non-waxy "normal" one in adults reflects different requirements of imagines and nymphs. The following reasons may be responsible:

- nymphs must be protected from wetting because they could drown if they got "trapped" by the surface tension of a water-droplet.
- the adhesion of contaminating particles on the surfaces of *Salvinia* and *Paulinia* is decreased by ultrastructural and chemical properties of their wax cover, which functions as a self-cleaning device (survey in BARTHLOTT 1990).
- a protection from wetting in young nymphal stages prevents their being washed off by strong water turbulences.

In contrast, adult females need to dive for oviposition, because egg-pods are attached on the underside of the plants and a hydrofuge cuticle would hinder them from that.

The change in cuticular morphology is complemented by different escape strategies: the cryptic, non-submergible nymphs rely completely on slow movements, while adults escape by jumping or submergence. This reminds on the different escape strategies in adult and young grasshoppers described by SCHULTZ (1981).

Feeding habits

The entire life of *Paulinia acuminata* takes place on its aquatic host plants. They feed on Salviniaceae like *Salvinia auriculata* and *Azolla filiculoides*, as well as *Spirodela intermedia*, *Hydromystria stolonifera* and *Pistia stratiotes* (CARBONELL 1964; VIEIRA & ADIS 1992). This is of economic interest because of its potential as a biological control of aquatic macrophytes as for example *Salvinia molesta* (SANDS & KASSULKE 1986; ROOM 1990). Oviposition was only observed on Salviniaceae and *Pistia stratiotes* (VIEIRA 1989). Ferns are considered to be an unattractive food for insects (HENDRIX 1980). However, there is quite a number of fern-eating Orthoptera. ROWELL et al. (1984) speculate that ancient groups of Orthoptera like the Eumastacodea may have coevolved with ferns since the Carboniferous, i.e. more than 200 million years. The association of *P. acuminata* with a fern could be a hint for a considerable age of the Pauliniidae. On the other hand, feeding experiments by VIEIRA (1989) showed that the fern *Ceratopteris* sp. is not accepted and the *P. acuminata* feeds on the above-mentioned angiosperms. Feeding behaviour of *P. acuminata* could therefore be an interesting paradigm to test present hypotheses which try to explain the low attractiveness of ferns for herbivores. BOPP (1988) suspects that a possible reason is the low

nutritive value of ferns; if this is correct, individuals feeding on *Salvinia* should have a greater food intake than those feeding on angiosperms.

Summary

The semi-aquatic grasshopper *Paulinia acuminata* (Orthoptera: Acridoidea: Pauliniidae) and its foodplant, the floating fern *Salvinia auriculata* (Filicatae: Salviniaceae) inhabit the surface of slowly running or stagnant neotropical waters (study material from French Guyana). Young grasshopper nymphs are well camouflaged by colour and surface structure which cannot be differentiated from *Salvinia* leaves. Relying on this mimetic protection, they hardly move when disturbed. Older nymphs and adult individuals which are bigger than the oval *Salvinia* leaves exhibit a somatolytic pattern and imitate two leaves with their interstitial reflecting water surface. Young nymphs as well as the leaves are extremely water repellent because of epicuticular waxes with similar ultrastructure based on functional analogy. Older nymphs and imagines which dive for temperature regulation and oviposition do not develop a wax layer and are wettable.

Zusammenfassung

Die semi-aquatische Heuschrecke *Paulinia acuminata* (Orthoptera: Acridoidea: Pauliniidae) und ihre Futterpflanze, der Schwimmpfarn *Salvinia auriculata* (Filicatae: Salviniaceae), leben an der Oberfläche neotropischer stehender oder langsam fließender Gewässer (Untersuchungsmaterial aus Französisch Guyana). Junge *Paulinia*-Nymphen sind in Farbe und Oberflächenstruktur von den *Salvinia*-Blättern kaum zu unterscheiden und bewegen sich mimetisch geschützt bei Störung kaum. Ältere Nymphen und adulte Individuen, die größer sind als die ovalen *Salvinia*-Blätter, zeigen ein somatolytisches Muster und imitieren zwei Blätter inklusiv reflektierendem Wasserzwischenraum. Junge Nymphen sind wie die Blätter extrem unbenetzbar durch epicuticuläre Wachse, deren funktionell bedingte Ähnlichkeit bis in den ultrastrukturellen Bereich reicht. Ältere Nymphen und Adulttiere, die zur Eiablage und Temperaturregelung abtauchen, entwickeln keine Wachsschicht und sind benetzbar.

Sumario

El saltamonte semi-acuático *Paulinia acuminata* (Orthoptera: Acridoidea: Pauliniidae) y su planta alimenticia, el helecho flotante *Salvinia auriculata* (Filicatae: Salviniaceae) viven en la superficie de aguas neotrópicas quietas o de lento movimiento. Las larvas jóvenes de *Paulinia* apenas pueden ser diferenciadas, en el color o en la estructura de su superficie, de las hojas de *Salvinia*. Debido a esta protección mimética, apenas se mueven cuando se los perturba. Larvas de más edad e individuos adultos, que son de mayor tamaño que las ovales hojas de *Salvinia*, presentan una estructura somatolítica imitando dos hojas con su reflejante espacio de agua intermedio. Las larvas jóvenes son, igual que las hojas, extremadamente repelentes al agua, debido a ceras epicuticulares, cuya similitud, condicionada funcionalmente, se extiende hasta la ultraestructura. Larvas de más edad y animales adultos, que salen del agua para desovar y para regular su temperatura, no desarrollan la capa de cera y no repelen el agua.

Acknowledgments

We are indebted to Manfred Koenen (Bot. Garden Univ. of Bonn) for supplying material of *Salvinia* and - incidentally - *Paulinia*, and to PD Dr. Joachim Adis, (Max-Planck-Institut für Limnologie, Plön) who has contributed most valuable aspects based on his long experience with terrestrial arthropods which experience seasonal flooding in the Neotropics. We thank Dr. Maria-Susana Cipolletti (Wasser) for the translation of the summary into Spanish.

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