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

by

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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 Salvinioaceae (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 overtops 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).

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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 wisk 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 aerolia 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 μm high and broaden slightly towards the base. Their mean distance is approximately 0.5 μm. Between the rodlets extraordinarily thin wax filaments, difficult to visualize even with high resolution SEM, having a diameter of 0.03 μm can be found. These crystallloid waxes occur on the leaf cells as well as on the trichomes.
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 ± 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.

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).
have investigated conditions of the fly plant surfaces.

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 & JEFFREY (1981) and SARGENT (1988) from the "white fly", an insect commonly found in green houses, and on cicada nymphs by MESSNER & ADIS (1992).

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-repellent 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 hydrophuge 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.

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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 get "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 hydrophuge 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 Salviniaeae like Salvinia auriculata and Azolla filiculoides, as well as Spirodela intermedia, Hydrorymista stolonifera and Pistia stratiotes (CARBONELL et al. 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 Salviniaeaeae and Pistia stratiotes (VIEIRA 1989). Ferns are considered to be an unattractive food for insects (HENDRIKS 1980). However, there is quite a number of fern-eating Orthoptera. ROWELL et al. (1984) speculate that ancient groups of Orthoptera like the Eumastacoidea 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: Salviniaeae) inhabit the surface of slowly running or stagnant neotropical waters (study material from French Guiana). 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

Sumario
El saltamontes semi-acuático Paulinia acuminata (Orthoptera: Acridoidea: Pauliniidae) y su planta alimenticia, el helecho flotante Salvinia auriculata (Filicatae: Salviniaeae) viven en la superficie de aguas neotropicas 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 ovalas 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 similaridad, 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.

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References


