Chapter 2
Easily Damaged Integument of Some Sawflies (Hymenoptera) is Part of a Defence Strategy Against Predators

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2.1 Introduction

During evolution insects have developed an impressive set of mechanisms to defend themselves against natural enemies. This includes adaptations in their phenology, behaviour, morphology, physiology, and chemistry (Evans and Schmidt 1990). Typically, at least two such traits occur jointly, so that one can speak about a real defence *strategy*. Yet, defensive traits per definition increase an insect's fitness, but taken alone one trait may be disadvantageous. A new kind of defence strategy was discovered recently in larvae of some sawflies (which are herbivorous Hymenoptera) of the family Tenthredinidae. So far unknown in other insects, it was called easy bleeding and defined as "a low mechanical resistance of the whole body integument, by which slight mechanical damage of the integument is enough to provoke the release of hemolymph at this given spot" (Boevé and Schaffner 2003; Fig. 2.1). By itself, this integument adaptation may be highly risky for the larva. Damaging the integument could lead to the loss of large quantities of hemolymph and, ultimately, to the death of the larva. Why does this not happen, and why, on the contrary, should the easy bleeding phenomenon be considered a defence strategy?
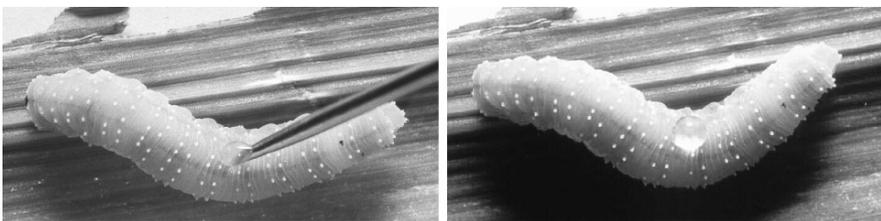

Fig. 2.1 The easy bleeding phenomenon. A larva of *Rhadinoceraea micans*, approximately 15–17 mm in length, is slightly touched on its body with a needle (*left*). A hemolymph droplet immediately appears at this spot (*right*)

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The present work addresses these questions and reviews our knowledge about easy bleeding. First, tenthredinid defence mechanisms are briefly described, especially those involving the integument (Part II). Next, several facets of easy bleeding are described (Part III). Then, adaptations of the integument are examined (Part IV) and the last part examines a novel use of the integument by one species (Part V).

2.2 Ecology of Tenthredinid Larvae

Tenthredinid larvae emerge from eggs usually laid within the plant tissue. The larvae are caterpillar-like and pass through four to eight instars, depending on species and sex, before moulting into a so-called prepupa. This instar may or may not feed, depending on species, and typically spins a cocoon in the soil. It will overwinter, then moult into a pupa in Spring, and emerge as an adult a few weeks later. More information on the life history can be found in Boevé (2008).

Tenthredinid larvae are herbivorous, usually feeding on leaves. The range of host plants is large, including mainly flowering plants, which grow as trees, shrubs and low plants, but also coniferous trees, ferns, etc. However, most tenthredinid species have a relatively narrow diet breadth; as specialists, they feed on one given plant genus or even plant species.

The majority of tenthredinid larvae live freely on a host plant leaf, but some groups of species, especially from the subfamily Nematinae, are endophytic (e.g., Nyman et al. 2000). Except for these latter cases, tenthredinid larvae are openly exposed to foraging predators, mainly arthropods, such as ants and bugs, and birds (e.g., Benson 1950).

There is growing evidence that under selective predation pressure, a large diversity of defensive mechanisms and strategies evolved among tenthredinid larvae. The integument often plays an essential role in these mechanisms. This is understandable since it is the body part, which as a boundary, is contacted first by an attacking predator. Thus, defensive allomones (i.e., harmful chemical compounds) are often emitted through the integument by specialized exocrine glands (Whitman et al. 1990). Moreover, the integument displays colour patterns, which may profoundly interfere with the visual cues of predators such as birds. The influence of the appearance of tenthredinid larvae in avian predator-prey relationships is detailed in Boevé and Pasteels (1985), Ohara et al. (1993), and Boevé and Müller (2005).

Exocrine glands are well documented in Nematinae larvae (Boevé and Pasteels 1985). Almost all species of this subfamily possess seven ventro-abdominal glands, which emit volatile compounds (e.g., Boevé et al. 2000). These compounds repel predatory arthropods such as ants from a distance (Boevé and Pasteels 1985). Each gland is composed of a layer of secretory cells, which form around a lumen, and a duct that opens to the exterior. The sac-like gland is everted by enhanced hydraulic pressure of the hemolymph, and retracted by the action of associated muscles (Boevé and Pasteels 1985). The cuticle of the secretory part is approximately $0.5\,\mu$m thick and is separated from the secretory cells by a sub-cuticular space (Boevé 1988). Globules contained in these secretory cells appear dense to electrons, and are most probably composed of precursor secretions. The secretion

reaches the gland lumen probably by diffusion and it will start evaporating once the gland is everted.

Larvae of *Caliroa* spp. are covered by a slimy layer and those of *Eriocampa* spp. by a waxy layer (Lorenz and Kraus 1957). In *Eriocampa ovata,* the wax is composed mainly of hexacosan-1-ol (Percy et al. 1983). In *Eriocampa babai*, wax filaments are especially long, forming cylindrical projections (Togashi and Nagase 1992). The following taxa possess a fine layer of waxy powder: *Eurhadinoceraea ventralis*, *Monophadnus spinolae*, *Monsoma* [= *Monosoma*] *pulveratum*, *Aglaostigma* and *Tenthredo* species (Table 2.1). Both *Caliroa* and *Eriocampa* larvae are well

Table 2.1 Occurrence of cuticle microstructures (CMS), hydrophobic property (HP), and appearance of integument (IA) in easy bleeders and non-easy bleeders belonging to the Tenthredinidae

Taxa	CMS	HP	IA
Easy bleeders (KPa < 600)			
Allantinae			
Athalia rosae	+	+	M
Blennocampinae, Phymatocerini			
Eurhadinoceraea ventralis	−	.	W
Monophadnus species B*[= *monticola*]	+	.	M
*Monophadnus monticola**[= *pallescens*]	.	.	M
Monophadnus spinolae	−	.	W
Phymatocera aterrima	+	+	M
Rhadinoceraea aldrichi	.	.	M
Rhadinoceraea bensoni	+	.	M
Rhadinoceraea micans	+	+	M
Rhadinoceraea nodicornis	+	+	M
Rhadinoceraea reitteri	.	.	M
Selandriinae			
Aneugmenus padi	+	−	M
Tenthredininae			
Pachyprotasis sp.	.	.	W
Non-easy bleeders (600 < KPa < 2,000)			
Allantinae			
Eriocampa ovata	.	.	W
Blennocampinae, Tomostethini			
Tomostethus nigritus	−	.	B
Heterarthrinae			
Caliroa cinxia	.	.	S
Nematinae			
Anoplonyx sp.	.	.	M
Craesus alniastri [= *varus*]	−	−	B
Craesus septentrionalis	−	−	B
Hemichroa crocea	−	.	B
Nematus bipartitus	.	.	B
Nematus caeruleocarpus	.	.	B
Nematus miliaris	−	.	B
Nematus pavidus	−	.	B
Pristiphora geniculata	.	.	B
Pristiphora laricis	−	.	B

Table 2.1 (continued)

Taxa	CMS	HP	IA
Pristiphora testacea	−	−	B
Selandriinae			
Strongylogaster mixta	−	−	B
Tenthredininae			
Aglaostigma discolor	.	.	.
Pachyprotasis sp.			.
Tenthredo bifasciata rossi	.	.	W
Tenthredo scrophulariae	−	.	W
Tenthredo sp.	.	.	.
Non-easy bleeders (KPa > 2,000)			
Allantinae			
Monsoma pulveratum	.	.	W
Nematinae			
Hemichroa australis	−	−	B
Hoplocampa testudinea	−	.	B
Selandriinae			
Strongylogaster multifasciata	−	−	B
Tenthredininae			
Aglaostigma alboplagiatum	.	.	W
Macrophya sp.	.	.	.
Tenthredo mandibularis	.	.	W

The taxa are distributed in three groups according to the pressure (KPa) needed to pierce the larval integument (Boevé and Schaffner 2003; Schaffner and Boevé, unpublished results). Cuticle microstructures present (+) or absent (−) (Boevé et al. 2004). Hydrophobic property relatively strong (+) or weak (−) (Boevé et al. 2004). Integument appearance matt (M) or brilliant (B), or integument covered with an obvious waxy (W) or a slimy (S) layer (Boevé and Schaffner, personal observations; Lorenz and Kraus 1957). Data unknown (.). Taxonomy (*) as in Prieto et al. (2007).

protected against foraging ants by their coverings (Eisner 1994; Boevé and U. Wyss, personal observations).

In several tenthredinid taxa, integumental structures are known to protect the larvae against predators. Relatively long and sometimes bi- or trifurcated bristles occur in the tribe Cladiini as well as in genera such as *Dineura* and *Periclista* (Lorenz and Kraus 1957). It is likely that the function of the body pubescence is to impede predatory ants from reaching and biting the prey's body (Boevé 1988), but it may also irritate birds when they seize a larva (Boevé et al. 2000).

Some tenthredinid larvae possess a particularly tough integument (Table 2.1). Toughness can be a consequence of the larval habit rather than a protection against predators (Boevé and Schaffner 2003). *Hoplocampa* larvae live and feed within fruits (apples, pears, etc). The first larval stage mines just under the young fruit epidermis, while further stages bore more deeply into the fruit. Thus one can expect the larval integument to sustain high frictional forces. The same reasoning applies to a species such as *Strongylogaster multifasciata* [= *lineata*] that lives freely on ferns, but pupates in a piece of tree bark. The larva will bore into this hard material and lodge in it. This again requires a tough integument.

2.3 Easy Bleeding: A Multi-Component System

Some tenthredinid larvae possess a very fragile integument that can be easily damaged (Part I; Fig. 2.1). Such species are called easy bleeders. We dissected a series of sawfly species, generally using ten specimens per species, and tested a piece of their integument for mechanical resistance (Boevé and Schaffner 2003). We measured the weight needed to pierce the fixed integument with a 0.4 mm diameter needle that manually and slowly moved perpendicular to the outer integument surface. The observed values, converted into pressure values, varied significantly among species, ranging from 164 to 4,653 KPa (Table 2.1). These data lead to the following conclusions. First, the values are a characteristic of the species, being reliably reproducible (for SD values, see Boevé and Schaffner 2003). *Rhadinoceraea aldrichi* has the lowest value measured so far. Second, the values of all species are distributed along a continuum (Boevé and Schaffner 2003). We therefore set an arbitrary limit at 600 KPa between easy bleeders and non-easy bleeders. This choice was made to include at least all Phymatocerini species which showed, using an empirical approach (see Fig. 2.1), to have an integument that is clearly easily damaged. This choice was supported by additional morphological data (Part IV). Third, the fact that any part of the integument, except the head, could be used for the measurements does reflect a major difference between the phenomenon of easy bleeding and the one of reflex bleeding. The latter occurs in several insect orders and is characterized by an integument that is easily damaged only at some localized places on the body, typically at an inter-segmental junction (e.g., Blum and Sannasi 1974). A last observation also underlines the difference between both phenomena: Reflex bleeding, unlike easy bleeding, does not need an exogenous, mechanical stimulus as an elicitor. *Athalia rosae* possesses several operculae at the end of the abdomen from which hemolymph can ooze (Hollande 1911). This species is also an easy bleeder (Table 2.1), thus exhibiting both phenomena.

Beside the interspecific variability of integument resistance encountered in Tenthredinidae larvae, an intraspecific variability also exists. During the moulting cycle of *A. rosae* integument resistance varies moderately, being lower shortly after moult and higher during pre-moult when compared to an inter-moult individual (Burret et al. 2005). In several species, the prepupa compared to its previous stage shows significantly lower values of integument resistance (U. Schaffner and Boevé, unpublished results).

So far, easy bleeding is known to occur in the following taxa (Table 2.1): *A. rosae*, *Aneugmenus padi*, *Pachyprotasis* spp. as well as all Phymatocerini tested (i.e., *Eurhadinoceraea ventralis*, *Monophadnus* spp., *Phymatocera aterrima* and *Rhadinoceraea* spp.). Genetic analyses of the Tenthredinidae revealed that these two species, one genus, and one tribe are not closely related to each other (G. Meijer, S.M. Blank and Boevé, unpublished results). Thus, easy bleeding appeared several times during the evolution of the Tenthredinidae and, as far as is known, it is restricted to this family.

Clearly, easy bleeding is a multi-component system. Besides the integument adaptation itself, several other adaptations co-occur. One of the key components

of the system is that the hemolymph emitted by easy bleeders is a feeding deterrent to several predators. Field observations revealed that easy bleeders are efficiently defended by a deterrent hemolymph when attacked by ant workers (Heads and Lawton 1985; A. Barker, personal observation) and wasps (Müller and Brakefield 2003). Easy bleeders are found on plants on which workers of ants such as *Myrmica rubra* are common. In the laboratory, bioassays with several types of predators showed that easy bleeding seems to be primarily directed towards insects with mandibles and less towards bugs (which attack by piercing) and vertebrates such as birds (Heads and Lawton 1985; Schaffner et al. 1994; Boevé and Schaffner 2003; Vlieger et al. 2004; Boevé and Müller 2005). Hemolymph extracts were tested on the ant *M. rubra* and the hemolymph of all easy bleeders tested so far proved to be moderately to strongly deterrent (Boevé and Schaffner 2003). Moreover, an inter-specific comparison showed that the more this activity is pronounced, the lower is the integument resistance. Such a significant association was interpreted as a functional link between both traits. This is a crucial point in understanding the function of easy bleeding as a whole. In a teleological way, one may say that the integument is easily damaged *because* the bleeding hemolymph is deterrent. When a predator bites into a larva, it will very rapidly come into contact with the hemolymph. This will shorten the attack and prevent a deeper and more serious wound (Boevé and Schaffner 2003).

The bioactivity of the hemolymph is caused by the presence of water-soluble micromolecules belonging to several chemical classes (steroid alkaloids, furostanol saponins, glucosinolates) and coming from the host plant. Such sequestration of secondary plant metabolites occurs in several species (*A. rosae*, *Monophadnus* spp. *P. aterrima*, *Rhadinoceraea bensoni*, *Rhadinoceraea nodicornis*) and the metabolites are strongly bioactive by a potent feeding deterrence (Schaffner et al. 1994; Müller et al. 2001, 2002; Boevé and Müller 2005; Prieto et al. 2007). Thus, the larva acquires, for its own defence, harmful plant compounds believed to have evolved for defence against herbivores. The hemolymph seems to be the elective site of sequestration since bioactive micromolecules were not detected in integuments, fat bodies and salivary glands of *R. nodicornis*, nor in integuments of *A. rosae* (Gfeller et al. 1995; C. Müller, unpublished results).

Once hemolymph has been released, there are several ways an easy bleeder can stabilize the quantities of remaining hemolymph. First, the larva can suck back any hemolymph droplet that remains on its body, provided it is still in contact with the hemocoel. This occurs within a few minutes (Boevé and Schaffner 2003; Burret et al. 2005). Second, the wound will heal. This occurs by local hemolymph clotting, formation of a scab and then melanization (A. Jakubowska and M. Spindler-Barth, personal communications). Third, easy bleeders are able to replace a relatively large quantity of hemolymph in the day(s) following its loss. Hemolymph was collected experimentally in a quantity equivalent to 10% of the larval body weight. In a comparison of untreated (control) and treated larvae of the same species, easy bleeders regained their normal weight within 24 hrs, whereas non-easy bleeders did not and these larvae had a low survival probability (Boevé, unpublished results).

Besides morphological adaptations of the integument and physiological adaptations such as sequestration of harmful compounds, wound healing, and replacing of hemolymph, there are also behavioural adaptations associated with easy bleeding. The normal behaviour of easy bleeders is to move slowly but, once disturbed, they become nearly immobile (Boevé and Müller 2005). This behaviour may have several advantages. Predators such as ants will not easily recognize the larva as a prey, since they are typically attracted by movements. For the same reason, the behaviour will shorten the attack if the larva is nevertheless bitten. Moreover, the larva prevents self-inflicted damage to its fragile integument by keeping movements to a minimum (Boevé and Müller 2005). Thus, it is likely that the mechanical property of the integument, in combination with ecological parameters, affects the general and defensive behaviours of easy bleeders.

2.4 Integument of Easy Bleeders: A New Kind of Functional Surface

Generally, the whole integument of easy bleeders, observed by scanning electron microscopy (SEM), is densely covered by typical microstructures (Boevé et al. 2004; Fig. 2.2). Their detailed appearance can vary from one species to another
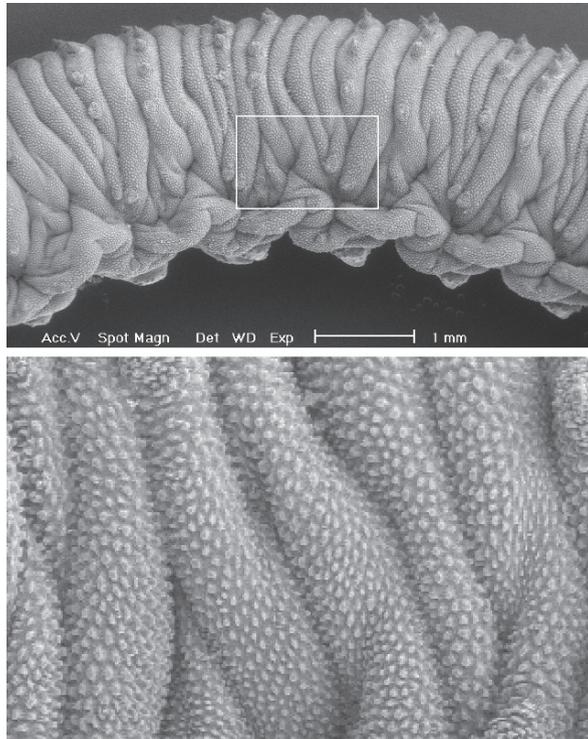

Fig. 2.2 SEM photos of a *R. micans* larva showing the cuticle surface and the microstructures which typically cover the whole body of easy bleeders. The picture above shows, in lateral view, several abdominal segments subdivided by annulets. The *inset* is magnified in the picture below

and from one part of the body to another. They are detected in the easy bleeders *A. rosae*, *Monophadnus* species B, *P. aterrima*, *Rhadinoceraea* spp. and *A. padi* (Table 2.1). They are absent in two easy bleeders, *E. ventralis* and *Monophadnus spinolae* (Boevé et al. 2004), but compared to the others, these two have higher integument resistance, ranging from 400 to 600 KPa (Boevé and Schaffner 2003). So far microstructures have never been observed in non-easy bleeders (Table 2.1). Thus, it is likely that they play a role in the phenomenon of easy bleeding. Noteworthy is that the arbitrary limit between easy and non-easy bleeders (see Part III) is largely reflected in the presence/absence of microstructures; and these structures are present in species from several rather unrelated tenthredinid subgroups (Table 2.1). This adds to the idea that the occurrence of microstructures is not necessarily linked to the phylogeny of species, but rather linked to the mechanism of integument disruption (see also below).

At a macroscopic level, the integument of easy bleeders tends to appear matt whereas that of non-easy bleeders as brilliant (Boevé et al. 2004). This pattern was confirmed for a series of species by checking the integument appearance of live larvae (Table 2.1). Thus, a matt integument is a first and rapid indication that a species may be an easy bleeder.

The external appearance of the larval cuticle is not the only morphological trait differentiating easy and non-easy bleeders. In almost all easy bleeders, an epicuticle could not be clearly detected by light microscopy of body sections stained with the Azan trichrome method (Boevé et al. 2004). Furthermore, the tri-dimensional arrangement and quantitative composition of the cuticle of easy bleeders appear different from those of non-easy bleeders when observed by transmission (and scanning) electron microscopy of KOH-treated larvae. In easy bleeders such as *P. aterrima* and *R. nodicornis*, chitin fibres form a loose network and the density of chitin fibrils is reduced and heterogeneous, whereas in the non-easy bleeder *S. multifasciata* layers of fibrils are densely packed (Burret et al. 2005).

After removal of the proteins from the cuticle of the easy bleeders *A. rosae* and *R. nodicornis*, SEM views revealed a polygonal pattern of groove lines on the inner surface of the cuticle (Spindler-Barth et al. 2005). A similar pattern was observed within the cuticle when integument sections of *R. nodicornis* were stained with a monoclonal N-acetylgalactosamine specific antibody. Most pore channels in the easy bleeder *Monophadnus* species B were also arranged in a polygonal pattern. Furthermore, phenoloxydase is present and tanning observed in easy bleeders. However, compared to non-easy bleeders, the cuticle of easy bleeders contains a higher concentration of soluble proteins, which may indicate a less efficient cross-linking of proteins. Such cross-linking determines, to a considerable extent, the cuticle stiffness in insects (Hepburn and Joffé 1976). In easy bleeders, it seems that impregnation with calcium replaces at least in part sclerotization. Indeed, Ca^{++} is detected by electron probe microanalysis in most easy bleeders, whereas it is not detected in most non-easy bleeders (Spindler-Barth et al. 2005). From all these observations, it becomes clear that the cuticle of easy bleeders shows particular adaptations in its (ultra)-structure. This raises questions about the extent and the way structural adaptations influence the mechanical properties of the cuticle, which in insects is

generally determined by many factors (Hepburn 1985). In easy bleeders, stiffness and hardness of the cuticle are probably provided by its impregnation with calcium that would, thereby, functionally replace sclerotization and render the cuticle rigid enough without increasing its resistance (Spindler-Barth et al. 2005). It is likely that this impregnation, in place of classical sclerotization, may allow the cuticle of easy bleeders to find a compromise in fulfilling two contradictory functions, as an exoskeleton while remaining easily damaged.

Furthermore, the microstructures may also be involved in the process of integument disruption. This assumption was based mainly on the observation that they generally occur only in easy bleeders (Table 2.1). A mathematical model, by finite elements, was developed to understand their potential influence during integument disruption (Boevé et al. 2004). Two cuticles were modelled, one without and one with a few microstructures (M1 and M2, respectively), representing the cuticle of a non-easy bleeder and an easy bleeder. When a normal (i.e., perpendicular) force is applied on a microstructure, the stress values obtained are similar in M1 and M2. But, if a shear (i.e., parallel) force is applied, the stress (i.e., compression and tension) values are approximately three times higher in M2 than M1. The same result was also obtained by incorporating into the modelled cuticle real values about the size of microstructures as well as the thickness of epi- and procuticles. Thus, the geometry of the microstructures, in its own right, is likely to facilitate the start of integument damage. The modelling also predicts that the break would go between the microstructures rather than through. Indeed, this type of break was observed when a fracture was provoked in the integument of the easy bleeder *P. aterrima*. This aspect of integument disruption is part of ongoing research using, among other methods, cryo-SEM.

A contradiction seems to exist between the results from the modelling and those from the resistance measurements of dissected integument pieces (see Part III). In the models, a significant difference between easy and non-easy bleeders was revealed only by applying a shear force, whereas in the bioassays it was revealed by applying a normal force. But, the two approaches consider very different orders of magnitude – one microscopic and the other macroscopic – and this may explain the observed differences. In a natural situation, a biting predator will most certainly apply a more or less oblique force on the cuticle, a situation not considered in either of the two approaches. However, due to the tip size of a worker ant's mandible, (a typical predator of easy bleeders), a load will be applied on only one or a few microstructures (Boevé et al. 2004). In this sense, the model is rather close to natural conditions.

When an easy bleeder is bitten, the droplet of hemolymph released will stay as a droplet on the body surface, due to the integument's hydrophobicity. The fact that the hemolymph does not spread has at least two advantages. It can be sucked back and does not need to be replaced. Furthermore, it remains spatially concentrated at the spot where the integument was pierced, leading to a good contact between the feeding deterrent hemolymph and the predator's mouthparts. Since the cuticle appeared to be involved in maintaining the hemolymph as a droplet, its hydrophobic property was analysed. The integument of easy bleeders, compared to that of

non-easy bleeders, proved to be more hydrophobic (Boevé et al. 2004; Table 2.1). It remains to be shown whether this is due to the occurrence of microstructures and/or the coating of microscopic particles. A waxy powder covers the whole body surface of several sawfly species, including easy and non-easy bleeders (Table 2.1). No waxy crystals at a micrometer scale were observed on the body surface of easy bleeders (Boevé and Müller 2005), but a more accurate analysis revealed their presence in *Rhadinoceraea micans*.

2.5 *Rhadinoceraea micans* as Case Study

R. micans is an easy bleeder that feeds on *Iris pseudacorus*. This plant lives along water (swamps, humid meadows, etc.), often having its rhizomes and lower aerial parts in water. The larvae of *R. micans* can move on the water surface, going from one plant to another in order to reach a new feeding site (Sägesser and Lüscher 1959). In the same manner, a larva that is experimentally dislodged and dropped from its plant can quite easily reach and crawl up another plant (Boevé, personal observation). These field observations were corroborated in the laboratory where the prepupal instar was used as a kind of control. This instar will sink once placed on the surface of water. A larva at last instar was taken between pincers and dropped from a height of 5 to 10 cm into a container of water. It came back to the surface. It was then taken again and pushed under the water. This was repeated five times, and it always returned to the surface. This experiment was repeated with ten larvae at last instar and ten prepupae. All prepupal individuals sank when dropped on the water surface (first experimental step). Thus, the integument of the larval stage proved to be highly hydrophobic, as indicated by previous tests (Boevé et al. 2004; Table 2.1).

In *R. micans* the tips of the microstructures are usually plateau-like enlarged (Fig. 2.3a). These broadened tips may contribute to decrease the contact area between the surface of the integument as a whole and the fluid, and thus, increase the hydrophobic property of the integument. A chemical factor may contribute as well to the property. Larvae freeze-killed and observed by SEM showed a diffuse matrix traversed by microscopic particles (Fig. 2.3b). Other specimens of larvae were fixed and stored in ethanol before gold coating. Micro-particles remained, whereas the matrix was no longer detected (Fig. 2.3c). The cuticle surface of prepupae differed completely from that of larvae, since in prepupae micro-particles, matrix, and microstructures were absent (Fig. 2.3d). Instead, the surface appeared "dirty" and was covered, among other things, by bacteria. We can conclude from these observations that the hydrophobic property of the larval integument in *R. micans* is probably due to both the particular microstructures, and the occurrence of a network of micro-particles that are embedded in a matrix. Discerning the relative importance of microstructure geometry and micro-particles plus matrix chemistry in causing this property remains under investigation.

Only a few other sawfly larvae than *R. micans* live on water-loving plants. The larvae of the tenthredinid *Dolerus vestigialis* feed on the water horsetail, *Equisetum*
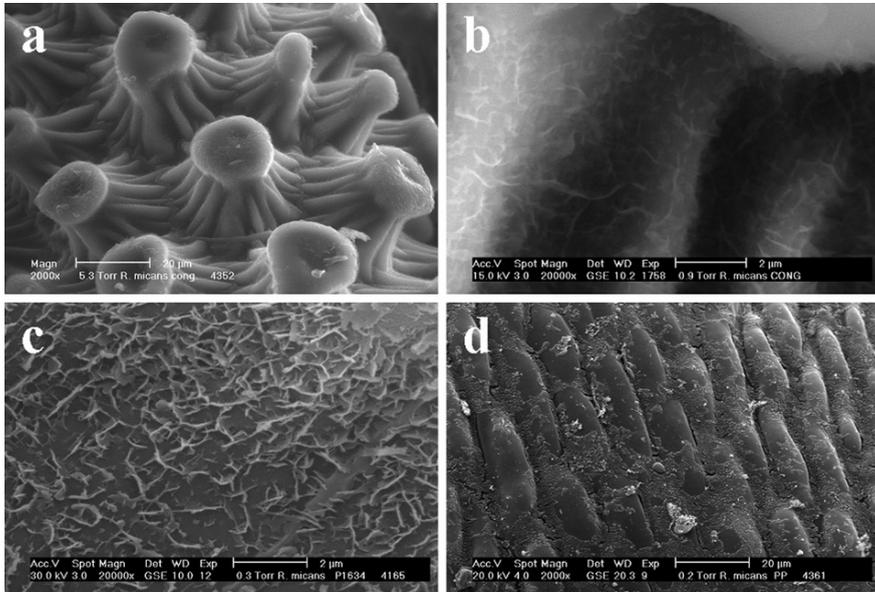
Fig. 2.3 Cuticle surface of *R. micans* observed by SEM. Larvae freeze-killed (**a–b**) or previously kept in a solvent (**c–d**). (**a**) Typical microstructures with enlarged tip; (**b**) matrix and micro-particles on the side of a microstructure; (**c**) micro-particles remaining after solvent treatment; (**d**) prepupa

fluviatile, and they were observed by Barker (2006) to be also able of moving on the water surface. Their integument, however, was not studied so far by SEM. Leblanc and Goulet (1992) show such SEM pictures for *Dolerus nitens* that feeds on terrestrial Poaceae, and they also mention the cuticle surface structure for six *Dolerus* species which feed on the field horsetail, *Equisetum arvense*. It seems that, the cuticle surface of all these *Dolerus* species strongly resembles the one of *R. micans* prepupae (as Fig. 2.3d). Thus, it remains unknown how *D. vestigialis* is able to move on the water surface, and whether its integument presents adapted microstructures and is as hydrophobic as the integument of *R. micans*.

One may suppose that the phenomenon of easy bleeding indirectly allowed *R. micans* to live in a semi-aquatic biotope. It is likely that adaptation to this new ecological niche, one not exploited by con-generic species, resulted from the evolution of the hydrophobic property of the integument, that was itself a result of the anti-predator defence strategy. This is an example of how a biological surface selected for one function was co-opted for use as another.

2.6 Conclusion

Easy bleeding was considered, first, as the capability for the body surface of some tenthredinid larvae to be easily damaged. This morphological trait may have been a preadaptation that allowed *R. micans* to live on a water plant. But, more generally,

the present review reveals that easy bleeding is a defence *strategy*, that is, it clearly includes behavioural, chemical, physiological, and morphological characters. It is this unique concomitance of characters that renders functional the anti-predator defence.

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