Defensive secretions from the larvae of *Apatania fimbriata* (Pictet) (Trichoptera: Limnephilidae)

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Summary

When the larvae of the caddis fly *Apatania fimbriata* (Pictet) are threatened, drops of fluid appear on their head capsules. The secretions are produced in a gland in the dorsal part of the prothorax. The neck region contains an eversible sac with numerous single setae, groups of 3 setae, or rows of setae on the surface. The secretion is released through two paris of orifices on the lateral sides of the sac. For the most part the secretion is composed of some 30 fatty acids, with the major components having 12–14 carbon atoms and up to 4 double bonds (approx. 1–2 μg secretion per specimen). Biotests with synthetic saturated acids (C_6–C_{12}) in a stream and in the laboratory demonstrated a paralysing effect on small invertebrate predators (*Rhyacophila* sp., *Plectrocnemia conspersa, Hydropsyche* sp., larvae, all Trichoptera). In choice experiments, *Rhyacophila* sp. larvae preferred larvae of *Agapetus fuscipes* and *Drusus annulatus* (Trichoptera) as food as compared with *Apatania fimbriata larvae.* Larger predators, such as *Dinocras cephalotes* (Insecta, Plecoptera) and the fish *Cottus gobio*, did not discriminate between *Apatania fimbriata* and other prey species. The use of fatty acids in defensive secretions is interpreted as an adaptation to the running water environment. They are effective repellents against *Rhyacophila* sp. larvae, the most important predator in the natural environment of *Apatania* larvae.

Key words

defensive secretion, cervical sac, fatty acids, prothoracic gland, Insecta, Trichoptera, Limnephilidae, *Apatania fimbriata*

Introduction

Pheromones and defensive secretions have been reported in the adults of a number of caddis fly species (*e.g.,* Duffield *et al.* 1977; Duffield 1981). In the past decades much research has been done on the sexually attractant pheromones of adult caddis flies (Kelner-Pillault 1975; Wood & Resh 1984; Resh & Wood 1985; Solem 1985, Resh *et al.* 1987). Probable "scent organs" were described by Eltringham (1919), Mosely (1923) and Roemhild (1980) for the genus *Hydroptila* Dalman. Pheromones and other odours play major roles in many important behavioural and physiological processes in insect life. In aquatic insects, differences have been found between the composition of the pygidial gland contents of various species of water beetles, and such differences have been investigated for chemo-taxonomic purposes (Dettner 1979). Many of these secretions were described as defensive substances used against microbes. Water beetles have been observed spreading the contents of their pygidial glands over their bodies to remove epizoic organisms.

During a life cycle study on the caddis fly *Apatania fimbriata*, one of the authors (M. A.) repeatedly observed drops of an oily fluid appearing on the larval head capsules. Seemingly these drops were secreted when the larvae felt endangered because they were observed during measurement of the head capsule, while larvae were being positioned with needles and tweezers. They withdrew into the larval case, turned their heads down and secreted drops of fluid. In some larvae, a translucent "cervical sac" arising from the front side of the prothorax was visible. Thus the area of the neck where the drops occured was presented to an enemy. A few seconds after the "attack" had ended, drops disappeared from the head capsule and the larvae tried to regain their normal position. Drops adhered easily to a pin and had a distinctive smell similar to that of train oil or fish oil.

Here, we examine possible reasons for the appearance of these drops, their chemical composition and their effectiveness as a chemical repellent against predators.

Material and methods

Transmission/scanning electron microscopy (TEM/SEM)

Apatania fimbriata (Pictet) larvae from the Breitenbach were used in all the studies mentioned below. The Breitenbach is a small Central European stream, situated near Schlitz (Hessen, FRG, 50°40′N, 9°45′E).

For TEM, larvae were fixed in glutaraldehyde in cacodylate buffer for 12 h, washed in 0.1 M/l cacodylate buffer overnight and dried in an acetone concentration series. Specimens were then embedded in vestopal (Wohlfarth-Bot-

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termann 1957). Sections were cut with glass knives on a Reichert ultramicrotome and studied with a Zeiss EM 9 S electron microscope at 60 kV.

For SEM, larvae were removed from their cases. The cervical sac was artificially everted by gently pressing the abdominal segments until the sac remained everted. It was fixed in a solution of glutaraldehyde in Soerensen buffer overnight, dehydrated in an acetone concentration series, and finally sputtercoated with gold. Photographs were taken with a Leitz AMR 1200 SEM.

Chemical analyses

Extraction of secretions – For the initial investigations the solution was injected directly into the injection port of the gas chromatograph together with a silylating agent. Later, secretions were collected with a syringe and subsequently diluted in methylene chloride.

Preparation of derivatives – All reactions were carried out in screw cap conical vials (0.1 to 1 ml). Chemicals were purchased from Merck, Darmstadt, FRG. Trimethylsilyl derivatives were produced on-line by injecting less than 1 µl of sample (corresponding to the content of two glands) together with an estimated 10 to 100 fold molar excess of silylating agent. N,O-bis-(trimethylsilyl)-acetamide (BSA) and N-methyl-N-(trimethylsilyl)-trifluoracetamide (MSTFA) were used for this purpose.

Positions of the double bonds were determined by interpreting the mass spectrometric fragmentation of the picolinyl (Christie *et al.* 1987) and dimethyl disulfide (DMDS) derivatives (Vincenti *et al.* 1987).

Picolinyl esters were prepared by a modification of the method of Harvey (1982). The diluted secretion of 10 glands (in ether) were dried in a stream of nitrogen. 10 µl N,N-dimethylformamide (0.1% in ethyl acetate) was added and the solvent evaporated. After adding 4 µl of thionyl chloride, the sample was allowed to stand for 25 min at room temperature. The thionyl chloride was then removed with nitrogen and 10 µl of 10% 3-pyridylcarbinol in ethyl acetate was added. 0.2 µl of this sample, together with 0.5 µl MSTFA to silylate the excess 3-pyridylcarbinol, was injected into the gas chromatograph.

To prepare dimethyl disulfide (DMDS) derivatives we produced methyl esters of the natural acids by reaction with an excess of freshly prepared diazomethane dissolved in ether (Leonhardt & DeVilbiss 1984). Samples containing the methylated secretions of 15 glands were then dried and 300 µl of DMDS and 30 µl of a 0.6% solution of iodine in ether were added. These were left to react for 15 min at 60 °C (Vincenti *et al.* 1987). After dilution with 400 µl of n-hexane, the reaction was stopped by adding 300 µl of a 5% solution of $Na_2S_2O_3$ in water. The organic layer was removed and evaporated to 10 µl. 0.2 µl of the sample was taken for analysis.

Gas chromatography-mass spectrometry – The secretions were analyzed with a Varian 3700 gas chromatograph equipped with a programmable temperature vaporizer (PTV) (Gerstel, Mühlheim), coupled directly to a Finnigan MAT 212 mass spectrometer. For data acquisition a Tekniv-ent Data System was used. Samples were introduced on a fused-silica DB-5 capillary column (60 m × 0.25 mm ID). GC operating conditions were as follows: column oven temperature: 2 min at 40 °C, programmed to 310 °C at 15 °C/min; carrier gas: helium at 1.5 ml/min. PTV: programmed from 40 °C to 300 °C at 10 °C/s. Ionization energy: 70 eV.

Biological tests

Biotests – Synthetic saturated organic acids (pure C_6 and C_8, and a mixture of C_6:C_8:C_{10}:C_{12} – 3:4:5:1 g) were used to test the reactions of potential predators in streams. Single drops (0.3–0.5 ml) of the synthetic acids (single or mixture) were released into the current some 10 cm upstream of some larvae. To observe the reactions of the larvae, stream regions with an unruffled water surface and the following approximate conditions were selected: current velocity 5–15 cm/s, water depth 5–10 cm. Drops of acid were released some cm below the water surface and were drifted to the test specimens by the current. Reactions of larvae after contact with the chemicals were described and noted. After the treatment 50% of the larvae were kept in plastic boxes in the stream and observed daily for survival. The others were maintained in the laboratory and observed 4 times a day, for 2 to 3 days, to determine their rate of survial. Predators tested in the stream were caddisfly larvae of *Hydropsyche* sp., *Plectrocnemia conspersa* Curtis, and *Rhyacophila fasciata* Hagen. The larval reactions were identical when mixtures of different fatty acids were used. For comparison larvae of *A. fimbriata* were placed in closed boxes with a 5% solution of fatty acids in stream water for 15 min, on 10 occasions.

Choice experiments – Choice experiments were conducted in gauze cages (20 × 10 × 10 cm) in the Breitenbach, or in a laboratory recirculating stream, in the autumn and spring. Cages were filled with stones from the Breitenbach with a visually intact biofilm. All macroscopic organisms were removed, although, if already present, the desired larvae were left on the stone surfaces. Otherwise they were introduced at this point. Prior to introduction of a predator, prey specimens were left for 1 day to settle on the stones.

Larvae of the caddis fly *Rhyacophila fasciata*, the stonefly *Dinocras cephalotes* Curtis, and the fish *Cottus gobio* L. were used as predators. Larvae of *Agapetus fuscipes* Curtis (Trichoptera, Glossosomatidae) and *Drusus annulatus* Stephens (Trichoptera, Limnephilidae, Drusinae) were compared with *Apatania fimbriata*. One or two predators and differing numbers of prey specimens of two different species were used. Experiments were run for five days. Numbers of surviving specimens were noted after 3 and 5 days. Treatments without predators were run as controls. No specimens died in the control systems. Differences between preferred prey species were evaluated using Manly's Preference Index α, calculated for *Apatania* as

$$\alpha_{Apa} = \frac{\ln[(N_{Apa} - C_{Apa})/N_{Apa}]}{\ln[(N_{Apa} - C_{Apa})/N_{Apa})] + \ln[(N_x - C_x)/N_x]}$$

(Chesson 1978, 1983). N_{Apa} and N_x are the initial numbers of *Apatania* and the species being compared (x), respectively C_{Apa} and C_x are the numbers of specimens consumed by the predator. The index ranges from 0.0 to 1.0. A value of $\alpha_{Apa} > 0.5$ would indicate that *Apatania* larvae were more

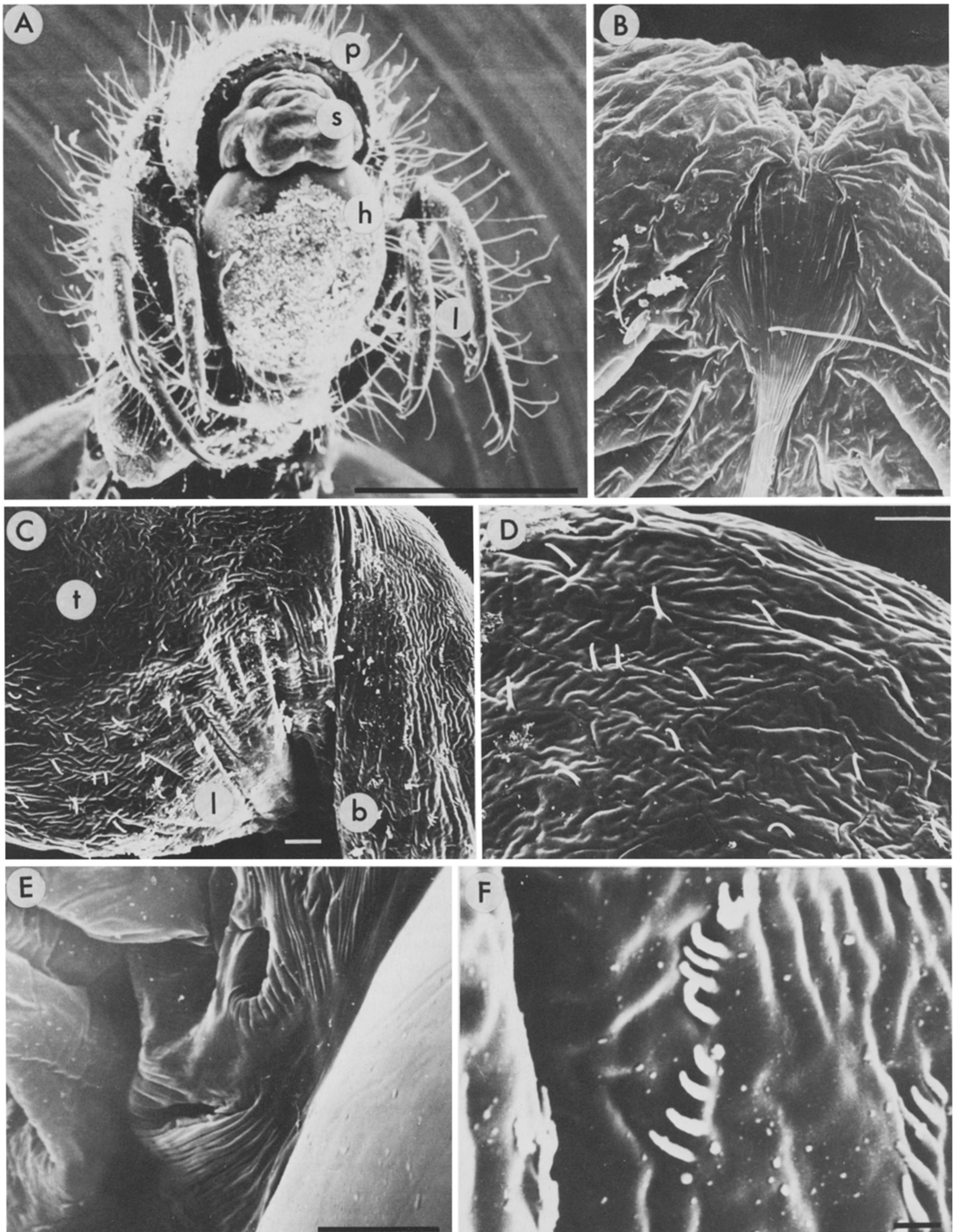

Fig. 1 **A** Front view of an *Apatania fimbriata* larva. h = head, s = cervical sac, p = pronotum, l = legs. **B** Chitinous band of the sac. **C** Dorsal view of the cervical sac. dorsal (t), lateral (l) and basal (b) region of the cervical sac. **D** Single micritrichium in front lateral area of the cervical sac. **E** Pair of openings through which secretions are released. **F** Basal region of the sac with rows of microtrichia. Scale bars: A 0.5 mm, B–E 10 µm, F 1 µm

vulnerable to a predator than the species being compared. α_x was calculated for each treatment and these values were used in a t-test with null hypothesis being $\alpha_x = 0.5$ (*i.e.* no difference in vulnerability) (Chesson 1983). Depending on the number of predators available, 3 to 6 replicates were run.

Results

Morphology of the cervical sac

The cervical sac arises from the front side of the prothorax. It consists of a thin uniform layer of chitin. The sac has two anterior and two lateral short projections (Figs 1A, 2A, B). The dorsal side is devoid of microtrichia (Fig. 1C), while there are numerous single microtrichia on the lateral and front sides (Fig. 1D,). Groups of 3 microtrichia occur in the central and lateral area whereas rows of up to 12 occur in the basal area (Fig. 1C, F). The morphology of these, which lack a basal ring, indicates that they probably do not act as chemosensilla. They appear to help keep the sac in place. A pair of small openings, 5 µm in diameter, is situated on the lateral sides near the base of the cervical sac (Fig. 1E). The secretions of the prothoracic gland are released and absorbed through these, probably by actively changing the pressure of the hemolymph. At the lower end of the sac, there appears to be a cuticular band that normally retains the sac on the front side of the prothorax and prevents overexpansion (Fig. 1B).

Morphology of the prothoracic gland

The gland lies in the front part of the prothorax, just behind the cervical sac (Figs 2A, B). The eversible neck membrane seems to be part of the gland reservoir. The morphology and anatomy are similar to that of other insect prothoracic glands with a simple reservoir (Forsyth 1968). The gland is horseshoe-shaped and situated around the front part of the gut, extending to the notum. However, there are two groups of secretory cells, one on each side (Fig. 2C). Evidently these cells produce the fatty acids (cf. below) and release them into the gland reservoir, as could be seen in several TEM photographs.

Excretions are released into the lumen of the gland, which, as in other insects, probably acts as part of a reservoir. We assume that changes of the reservoir's volume are caused by either changes of the volume of the cervical sac and/or changes of hemolymph pressure. Thus, the secretion is either pressed out through the two pairs of openings on the lateral sides of the sac, or it is 'sucked back' into the sac due to cohesive forces of the secretion molecules. The secretion was observed to disappear after an attack was finished, before the larvae regained their normal positions in the case and then on the substratum. Thus a single unsuccessful attack did not use up the entire amount of defensive secretion available. This may be of great importance. If larvae needed several hours to replenish the gland reservoir they were more vulnerable to predator attack during that period.

Chemical analyses

GC-analysis of the trimethylsilyl derivates of the prothoracic gland content revealed a complex composition of saturated and unsaturated acids. More than 30 acids were detected (Table 1). Quantification with an external standard of trimethylsilyl esters showed that the dominant compounds (*cf.* Fig. 3, peaks 24, 28, and 35) were present at concentrations of 1–2 µg per specimen. Probable structures for the unsaturated acids are given in Table 1. Some branched acids were also found but these occurred in low concentrations and will be the subject of future investigations.

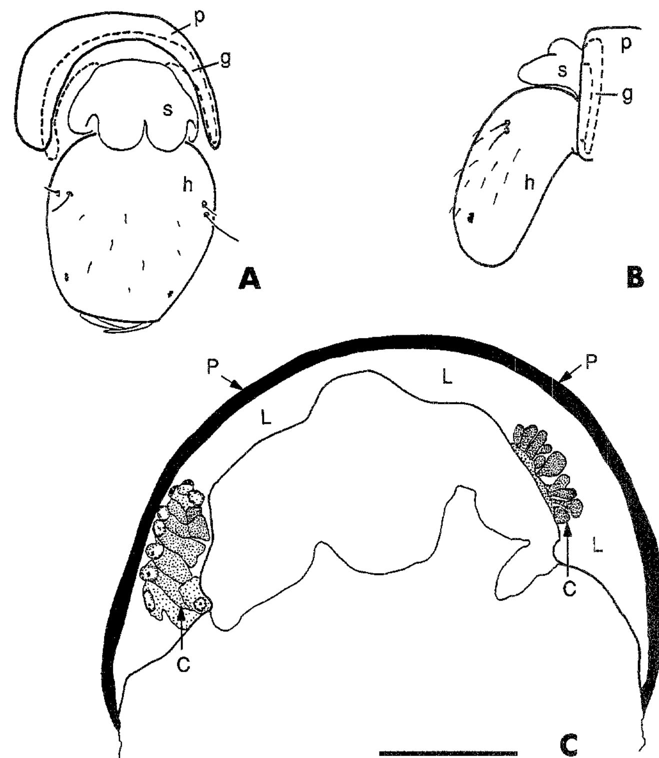

Fig. 2 Schematical drawing of an *Apatania fimbriata* larva: **A** Front view and **B** lateral view of h = head, s = cervical sac, p = pronotum, and g = gland (broken line). **C** Cross-section of the pronotum with gland, p = pronotum, c = glandular cells, l = lumen of gland reservoir. Scale bar: 0.3 mm

Table 1 Acids identified from the prothoracic glands (see Fig. 3) of *Apatania fimbriata* larvae

Peak #	Acid	Peak #	Acid	Peak #	Acid
1	n-C7:0	13	C10:1, Δ^{9}	25	n-C12:0
2	C7:0	14	C10:1	26	C12:2
2a	n-C7:0	15	n-C10:0	27	C12:4
3	C8:0	16	C11:1	28	C12:2, $\Delta^{3,5}$
4	C8:1, Δ^{5}	17	C11:0	29	C12:3, $\Delta^{3,5,9}$
5	n-C8:0	18	C11:0	30	C13:2
6	C9:0	19	C11:1	31	C14:3
7	C9:0	20	C11:1	32	n-C14:0
8	C9:1	21	n-C11:0	33	C14:4
9	n-C9:0	22	C12:2	34	C14:3, $\Delta^{3,5,9}$
10	C10:0	23	C12:3	35	C14:4, $\Delta^{3,5,7,11}$
11	C10:2	24	C12:1, Δ^{3}	36	C14:2, $\Delta^{3,5}$
12	C10:1, Δ^{3}				

C10:2 = 10 C-Atoms: 2 double bonds, superscripts indicate positions of double bonds

Biotests with predators in streams

The highest sensitivity to fatty acids was observed with larvae of *P. conspersa*. They detached themselves from the substratum immediately after contact with the pure

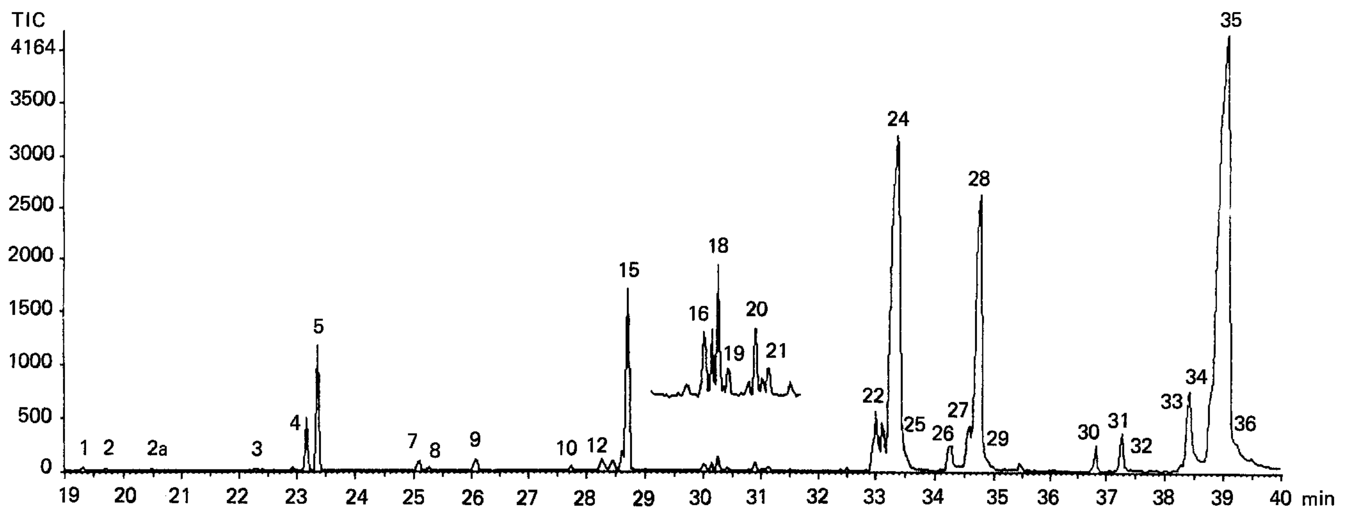

Fig. 3 Gas chromatogram of a silylated secretion from the prothoracic gland of an *Apatania fimbriata* larva (peak identification refers to Tab. 1)

or the above mentioned mixture of fatty acids released into the stream water. Their bodies shook for a few seconds and, having lost contact with the substratum they drifted away. Normal reactions, such as being able to maintain contact with the substratum were no longer observed. All the larvae treated with fatty acids were paralysed and died within a few minutes or, at the latest after a few hours. Larvae kept in gauze cages in the laboratory under conditions similar to the stream were also unable to recover, with none of the 12 larvae treated in this way surviving. These effects were not simply due to the depression of pH, because *P. conspersa* larvae are relatively insensitive to low pH levels. They are one of the few species to occur in streams of pH 5 or less.

The response of *R. fasciata* larvae on the same compounds was different. Some tried to remain attached by clinging tightly to the stone surface, while others pressed their abdominal segments and gills to the substratum and raised their thorax regions, shaking and nodding with the front parts of their bodies. However, in the end they all crawled backwards underneath the stones and tried to remain in place, retaining this position for at least 10 minutes. Most animals showed no response when removed from the substratum. They were unable to reattach themselves and began to drift. When checked a day later, both in the laboratory and in the stream, their mobility and other behaviour had returned to normal. Only one of 15 specimens treated died. Thus although behavioural changes were initially as dramatic as in *P. conspersa*, almost all the specimens recovered from exposure to the fatty acids within a few days in running water.

Hydropsyche larvae were also vulnerable. Specimens treated with fatty acids lost their ability to cling to the substratum. At this point they did not start to roll up as usual when endagered. They stretched their bodies instead. Larvae treated with single drops of the above mentioned pure acids and the mixture recovered within 20 minutes, others treated with several drops needed between several hours and one day for complete recovery. Responses were similar to those of the above mentioned species, and only one specimen of the 10 treated died.

Qualitative differences between the pure fatty acids and the mixture, concerning the effects on the predators mentioned above were not detected. Larger quantities released into the stream water intensified the observed reactions, and finally killed the target organisms.

A. fimbriata larvae placed in closed boxes with 5% solutions of fatty acids (pure and mixture, see above) in stream water for 15 min remained in their normal positions, crawling over the substratum surface and carrying on feeding. With the same treatment, *Rhyacophila* larvae immediately began shaking their bodies, as described above.

Food selection experiments

Rhyacophila larvae were never seen attacking prey because this species is nocturnal (Knöfel 1983). This predator never destroyed the caddis cases in these experiments. The cases of *Agapetus fuscipes* remained entirely intact but larvae were nevertheless killed. The predator probably invaded from the front, or attacked the neck region. This was evident from the larval remains. Only the head capsules of *Agapetus* were found, and *Drusus* larvae were almost entirely eaten. In the few cases where *Apatania* larvae were killed, the head capsule was bitten off but the thoracic sclerites and the rest of the body remained in the case.

Rhyacophila larvae preferred larvae of *A. fuscipes* and *D. annulatus* in choice experiments in the field and in the laboratory (Table 2). Vulnerability of *A. fimbriata* larvae was significantly lower compared with the other species. Results remained the same when twice as many *Apatania* larvae were offered compared with both other species (laboratory) or when two predators per treatment were used (field). In the only experiment when no larvae were preyed upon the *Rhyacophila* larva moulted.

Other predators, laboratory experiments

With the plecopteran *Dinocras cephalotes* larvae as predator, the cases of *A. fuscipes* were broken. Cases of *D. annulatus* were also broken, or at least partially de-

Table 2 Laboratory and field preference tests, predator *Rhyacophila fasciata*, prey organisms *Apatania fimbriata Agapetus fuscipes, Drusus annulatus* (id = intital density per treatment, fd = final density per treatment, numbers indicate the amount of parallel treatments, s = sum of id and fd, * = p<0.001, ** = p<0.0001, t-test, Manly's preference index against h_0 = equal vulnerability)

Laboratory experiments

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Agapetus*
id	1	10	10
fd_1	1	10	2**
fd_2	1	10	7**
fd_3	1	10	5**
fd_4	1	10	4**
fd_5	1	10	5**
s_{id}	5	50	50
s_{fd}	5	50	23

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Agapetus*
id	1	20	10
fd_1	1	20	6**
fd_2	1	19	3**
fd_3	1	20	5**
s_{id}	3	60	30
s_{fd}	3	59	14

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Drusus*
id	1	10	10
fd_1	1	9	5**
fd_2	1	10	6**
fd_3	1	10	10 ns
fd_4	1	10	7*
fd_5	1	10	8**
fd_6	1	10	6**
s_{id}	6	60	60
s_{fd}	6	59	42

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Drusus*
id	1	10	5
fd_1	1	10	2**
fd_2	1	10	1**
fd_3	1	10	2**
fd_4	1	10	2**
s_{id}	4	40	20
s_{fd}	4	40	7

Field experiments

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Drusus*
id	2	7	7
fd_1	2	7	2**
fd_2	2	7	4**
fd_3	2	7	2**
fd_4	2	7	3**
s_{id}	8	28	28
s_{fd}	8	28	11

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Agapetus*
id	2	10	10
fd_1	2	10	1**
fd_2	2	10	0**
fd_2	2	10	2**
s_{id}	6	30	30
s_{fd}	6	30	3

	predator *Rhyacophila*	prey 1 *Apatania*	prey 2 *Agapetus*
id	2	10	10
fd_1	2	10	1**
fd_2	2	10	0**
id	2	20	20
fd_1	2	18	7**
fd_2	2	20	7**
fd_3	2	15	0**
fd_4	2	18	2**
s_{id}	12	100	100
s_{fd}	12	91	17

stroyed, mainly at their front margin. The prey was probably dragged from its case before being consumed, as also observed in nearctic stoneflies (Nislow 1991). In the few cases where *Apatania* larvae were preyed upon, the case was broken at the rear end. This appeared to be similar to preying behaviour described by Gislason (1981) for the larvae of *Potamophylax cingulatus* Stephens (Trichoptera). This author reported cases of *Apatania zonella* Zetterstedt (Trichoptera) being mainly ripped by the predator from the ventral side. Thus the predator probably would not have any contact with secretions on the front, dorsal side of the body.

In experiments using *Cottus* as a predator, cases of all species were destroyed by chewing movements of the fish. The secretion was apparently ineffective against fish and large plecopteran predators.

Discussion

Chemical analysis

The variety and quantities of the mostly unsaturated fatty acids in secretions from the prothoracic glands of *A. fimbriata* larvae are the surprising results of this investi-

Table 3 Laboratory experiments for prey preference of *Dinocras cephalotes* and *Cottus gobio* with the caddis flies mentioned in Table 2 (for statistics refer to Table 2)

	predator *Dinocras*	prey 1 *Apatania*	prey 2 *Agapetus*		predator *Dinocras*	prey 1 *Apatania*	prey 2 *Drusus*
id	1	10	10	id	1	10	10
fd_1	1	10	8**	fd_1	1	10	8**
fd_2	1	8	3**	fd_2	1	8	1**
fd_3	1	9	10 ns	fd_3	1	9	8 ns
fd_4	1	9	7 ns	fd_4	1	9	7 ns
S_{id}	4	40	40	S_{id}	4	40	40
S_{fd}	4	36	28	S_{fd}	4	36	24
	predator *Cottus*	prey 1 *Apatania*	prey 2 *Agapetus*	predator *Cottus*	prey 1 *Apatania*	prey 2 *Agapetus*	
id	1	10	10	id	1	10	10
fd_1	1	1	3 ns	fd_1	1	2	0 ns
fd_2	1	2	0 ns	fd_2	1	0	1 ns
S_{id}	2	20	20	S_{id}	2	20	20
S_{fd}	2	3	3	S_{fd}	2	2	1

gation. An unusually high degree of unsaturation among the short-chained acids led to the application of several methods for determining the positions of the double bonds. One of these methods is the preparation of the picolinyl esters, a remote site derivatization introduced by Harvey (1982). Normally this reaction gives quantitative yields without side reactions. Because of the characteristic fragmentation pattern of these compounds the double bond positions generally may be deduced in mono-unsaturated acids. The application to di- and poly-unsaturated compounds is sometimes restricted. But even in this case many isomeric compounds can be excluded.

The structural analysis of mono-unsaturated compounds by their DMDS derivatives gives evidence for the original double bond position. Different reaction pathways must be considered for di-unsaturated compounds (Vincenti *et al.* 1987). The method fails for higher unsaturated compounds. Combining the results obtained from the mass spectra of both derivatives, a large number of possible isomers could be excluded.

Cervical sac and gland

So far as is known all species of the genus *Apatania* Kolenati appear to possess a cervical sac and probably use defensive secretions (JO Solem pers. comm. for Scandinavian species, and personal observations). Further, species of the closely related genus *Apataniana* Mosely also have a cervical sac. It is Y-shaped, with significantly longer distal prolongations than in *Apatania* larvae (Mey & Levanidova 1989).

With regard to its position, the cervical sac is analogous to the osmaterium of various lepidopteran caterpillars (Weber 1974). Prothoracic glands are known in caterpillars of various lepidopteran families (Blum 1981, 1987). The cervical sac of *Apatania* larvae is not actively evertible like the osmaterium of, for example, the Papilionidae. In the few cases where a specimen was found with an everted sac, it could not be actively drawn back into its normal position close to the front side of the prothorax. We suppose the chitinous band had been destroyed. This assumption was also based on the observation that when a specimen was forced to evert its sac by gently pressing the front segments of its abdomen, the sac volume decreased when the pressure ceased.

Predator-prey interaction in a running water environment

Aquatic predator-prey interactions have been reviewed recently by Allan (1983), Peckarsky (1984) and Kerfoot & Sih (1987). Feeding has generally been considered opportunistic and proportional to food or prey availability. However, recent research has produced evidence that predators can use a number of different cues to detect appropriate prey, and can discriminate between prey species and prey sizes (Allen *et al.* 1987; Williams 1987; Warren & Lawton 1984; Walda & Davies 1985; Peckarsky & Wilcox 1989). On the other hand prey species have developed species specific strategies to minimize the risk of predation (*e. g.*, Peckarsky 1985, 1987; Hershey & Dodson 1987; Williams 1987).

Comparing the food consumed with food availability has generally indicated a heavy utilization of the invertebrate fauna by predators, although removal of major predators in the field did not cause any change in the stream community (Allan 1983). In laboratory studies, the presence of two predators (*Cottus bairdi* and the perlid stonefly *Agnetina capitata* Pictet) revealed that significanlty fewer *Baetis* larvae were consumed in the presence of both, compared with model predictions of negative interactions between predators (Soluk & Collins 1988). However, a higher feeding rate of *Cottus* on larvae of *Ephemerella* was observed in the same experiment.

The use of defensive secretions has been documented for example, in gyrinid and dytiscid beetles. They release substances from their pygidial and thoracic glands that have narcotic or toxic effects, even on fish (Dettner 1979, Peckarsky 1984, and references therein). There is some controversy as to whether the use of these chemicals represents a primary or a secondary defense, with the latter being used only when a predator is encountered.

In the case of *A. fimbriata* larvae, a secondary defense is assumed. The secretions are released only in the case of a serious attack, when fastening the case to the substratum and withdrawal into the larval case failed to deter the predator, and the predator invaded the case.

All predators and prey organisms of the present study inhabit central European mountain streams. All the prey organisms are grazers, with larvae feeding on the epilithic biofilm. They are thus restricted to the running water environment, and cohabit on the gravel and stone substrata. Predators such as *Cottus* and *Dinocras* are rare, or even absent in stream sections inhabited by *Apatania* larvae. This is probably because of this species' large body size, which is a disadvantage in streams with water depths of often less than 2 cm. In such an environment the predators' mobility would increase their vulnerability to species from the terrestrial environment, such as birds like the water ouzel (*Cinclus* cinclus L.). The only notable predators of *Apatania* larvae among all the species present in their natural environment are larvae of the caseless caddis fly genus *Rhyacophila* (H. Malicky, pers. comm.).

We never witnessed an attack because of the nocturnal behavior of *Rhycophila* larvae (Knöfel 1983), but we also never found a single destroyed case of any prey organism. We assume the predator attacks its prey in the neck region while it is feeding or constructing its case. The neck region of *Apatania* larvae is better protected than that of *Agapetus* and *Drusus* due to the shape of its case. The front edge slants distally so that only part of the head capsule is visible.

If a *Rhyacophila* larva attacks, it presumably enters the case from the front. In response, the *Apatania* larva withdraws into its case, turns its head capsule down and presents its neck region with the secretions towards the invader. The mouth parts and adjacent sensillae of the predator are probably exposed directly to the secretion, inducing it to leave the case. *Agapetus* larvae leave their cases when endangered which is likely to make them an easy victim for a predator. Thus the interrelationship between larval behavior and the use of secretion is clearly demonstrated.

This larval behavior utilizes small amounts of low polarity, hydrophobic fatty acids most, which are effective even in the running water environment. The secretions are used within the larval case, where there is little or no current. Drops are secreted only in the event of an attack, in which case the invader is effectively confronted with high concentrations of the secretion. The cohesive force of the secretions and their use within the case are effective in two ways: the secretion cannot be washed away entirely by higher currents in the stream outside the case, and any secretion remaining unused after an attack can be reabsorbed into the gland reservoir. This reduced the vulnerability of the larvae after a single attack. While collecting secretions for the GC-MS studies, we observed that 5–6 h were needed to replenish the entire supply of secretion.

The use of fatty acids is interpreted as an adaptation to the running water environment. Volatiles, sprays and jets, as well as large amounts of water soluble chemicals, would be ineffective in the running water environment. The current would rapidly disperse such material and leave the larvae without defensive substances for several hours. Further, microcurrents in the vicinity of the case's exterior would dissipate secretions unpredictably, rendering an effective and directed application against a predator almost impossible. Fatty acids are secretions which influence the entire body surface, as demonstrated by the field biotest. They should thus be even more effective when in contact with the predators mouthparts and antennae.

The longitudinal distribution of *Apatania fimbriata*, along the stream, with densities decreasing with distance from the spring (Aurich 1989), depends on the increase in predators, such as fish and stonefly larvae, which are not affected by the specialized larval behavior or the secretions. Evidence for this hypothesis is provided by Gislason (1981), who reported that predation by larvae of the caddis fly *Potamophylax cingulatus* excluded larvae of *Apatania zonella* from large areas of Iceland. *Apatania* cases were ripped along the ventral side. Only the soft parts of the larvae (mainly abdomen) were consumed, suggesting that this predator did not contact the thorax region or the secretions of *Apatania* larvae. The conclusion was that *P. cinculatus* limits the distribution of *A. zonella* by predation.

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