Cantharidin-Secretion by Elytral Notches of Male Anthicid Species (Coleoptera: Anthicidae)

Claudia Schütz and Konrad Dettner

Lehrstuhl für Tierökologie II, Postfach 101251, NWI, D-W-8580 Bayreuth, Bundesrepublik Deutschland

Z. Naturforsch. **47c**, 290–299 (1992); received September 13, 1991/January 12, 1992

Cantharidin, Elytral Notches, Sexual Selection, Anthicidae, Coleoptera

Within the beetle family Anthicidae or ant-like flower beetles the canthariphilous species *Notoxus monoceros* shows a pair of notch-like structures on the apices of male elytra. A reservoir-like swelling at the apex of each elytron contains a bilobed gland. Its glandular tubules run into the sclerotized notch. Gaschromatographic investigations of elytral notches and the testes of cantharidin-fed and unfed male *Notoxus monoceros* revealed huge quantities of cantharidin stored in these organs in fed specimens. Other body parts, such as legs, elytral parts or flight muscles, had low cantharidin-titres, both in cantharidin-fed and unfed beetles. The cantharidin-titres of unfed individuals were very low compared with cantharidin-fed specimens, although cantharidin was present, mostly in notches. During sexual display the females bite several times into these notches and easily copulate, if the males had access to offered cantharidin crystals. Thus, the elytral notches of male *Notoxus monoceros* seem to function as „test-organs" for females which are able to select males with high cantharidin loads prior to copulation. Even small quantities of cantharidin stored in notches of field caught specimens seem of advantage for males in competing for a female partner.

Introduction

A number of anthicid species (Coleoptera: Anthicidae, ant-like flower beetles) are attracted by cantharidin in the field, and trapped specimens readily devour offered crystals [1]. Cantharidin trapping experiments in Tanzania, Kenya, Jugoslavia and Spain were used to study this insect-cantharidin interaction and to add to the known number of canthariphilous Anthicidae species of certain tribes and genera of the subfamilies *Anthicinae* and *Pedilinae* compiled from literature data. It is striking, that in most cases only males are attracted by cantharidin. Furthermore, these males often show a peculiar notch-like structure on the apices of the elytra. In 1954 Van Hille [2] described these notches as an elytral sense organ, which could be responsible for the attraction of male anthicid beetles to cantharidin. Our observations of the sexual behaviour of *Notoxus monoceros* indicated that the female was strongly attracted to the elytral notches of the male. Before accepting a male for copulation the female licks at the apex of the notched elytra of the male several times. In the laboratory it was obvious that only cantharidin-fed male specimens were accepted by females for copulation. No copulation attempts were observed with unfed male individuals, indicating that these notches may play an important role in cantharidin-manipulating male anthicids.

The aim of this investigation was to study the morphological structure of these peculiar elytral notches and to determine which canthariphilous anthicid species possess this organ. Furthermore, the function of the notch in the biology of canthariphilous species and the role of cantharidin for these beetles were investigated.

Reprint requests to Prof. K. Dettner.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/0300–0290 $ 01.30/0

Methods and Materials

Biological material. Male specimens of *Notoxus monoceros* were caught in traps with cantharidin baits in Karlsruhe (southwest Germany) and Bayreuth (southeast Germany) during the first two weeks of April 1991. A piece of filter paper soaked with a cantharidin-acetone-solution was hidden under gauze, so that an intake of cantharidin by the beetles was not possible.

In the laboratory 12 specimens (3 from Bayreuth, 9 from Karlsruhe) were immediately frozen, while 12 beetles from Karlsruhe were given the opportunity to feed continously on cantharidin crystals for 2–4 days before being frozen (8 individuals four days, 2 three days and 2 two days).

Morphological analyses. Elytra of numerous specimens were macerated in a 2.5% KOH solution for 24 h. The inner elytral membrane was removed and the elytral apex was prepared for scanning electron microscopy (SEM; Cambridge S 90, film: Agfapan 25).

Chemical analyses. For each specimen both elytral apices, an elytral fragment, one testicle and in several cases a leg (middle leg with tarsus, tibia, femur and trochanter) and flight muscles were isolated. Fresh weights of the cuticular fragments were registered (Sartorius semimicro balance R 200 D) except for the testicles and the muscles. From one of the paired testes and flight muscles of each individual the fresh and dry weights were determined. For five specimens the dry weights of the elytral parts were established as well as the fresh weights.

The beetle fragments were put into injection needles (mini-injector 10 µl, Hamilton) and injected into a Carlo Erba capillary gas chromatograph (Vega Series 2, 25 m FS-OV 1701 glass capillary column; temperature program: 80 °C to 300 °C with a heating rate of 15 °C/min; carrier gas: helium). To determine the cantharidin-titre of the field caught and cantharidin-fed specimens two calibration curves were produced with two standard solutions at different attenuations (SGE, microliter injection needle, 1 µl).

Cantharidin GC-peaks were integrated (Spectralyzer, spectra-soft) and expressed as ng cantharidin/µg body fresh weight (w). Statistical procedure (t-test) were conducted to test whether the results were different from each other (* = $p < 0.05$, significant; ** = $p < 0.01$, highly significant; *** = $p < 0.001$, very highly significant).

Results

Presence of notches within canthariphilous anthicid-beetles – a literature survey

Of the worldwide-distributed family of Anthicidae with about 3500 species 148 species belonging to 18 genera (subfamily *Anthicinae* and *Pedilinae*) are recorded to be canthariphilous (Table I). Within 5 genera of this compilation notch-like structures situated on elytral apices are present in the males *(Notoxus, Mecynotarsus, Aulacoderus, Microhoria, Tenuicomus)*, and 11 genera are without these structures *(Acanthinus, Anisotria, Anthicus, Endomia, Formicilla, Formicomus, Hirticomus, Pseudoleptaleus, Pseudonotoxus, Sapintus, Vacusus)*. For two genera *(Pedilus, Tomoderus)* neither literature data nor collected material was available, but in no case is it known from morphology that any species of these genera is in possession of such a structure. Another two genera are known to have notches in the male *(Hypaspistes, Clavicomus)*, but no canthariphilous species has yet been recorded (personal communication of G. Uhmann).

Tribe Notoxini of subfamily Anthicinae [14]

The genera *Notoxus, Pseudonotoxus, Plesionotoxus, Mecynotarsus* and *Hypaspistes* belong to the tribe *Notoxini* of the subfamily Anthicinae and are additionally characterized by a pronotal horn (Fig. 1). For the genera *Notoxus* and *Mecynotarsus* canthariphilous species with notches on the elytra of the males are recorded. In the genus *Hypaspistes* the presence of notches seems questionable but no canthariphilous species is known. In the genus *Pseudonotoxus*, at least *P. testaceus* is without notches [15], whereas no data are published about *Plesionotoxus*. Thirty one species from 58 listed *Notoxus* species in Table I show notches, whilst for 27 species no literature data concerning presence of notches were found. In most cases only male individuals can be baited with cantharidin. The species *Notoxus trifasciatus* lacks elytral notches and shows no attraction to cantharidin. Six species of *Mecynotarsus*, of which one species is known to have a notch, are attracted to cantharidin; for five species no data could be collected. *Pseudonotoxus testaceus*, was found at a cantharidin bait as well, although this species does not possess elytral notches [15].

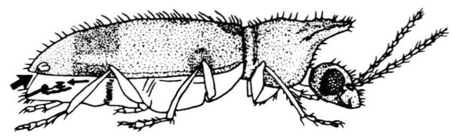

Fig. 1. Side view of male *Notoxus monoceros* with elytral notch and associated gland (short arrow). Internal male sexual organs are indicated by a lengthened arrow.

Table I. Compilation of canthariphilous Anthicidae species including data on presence/absence of notches and attracted sexes (m: male; f: female, –: absence, ?: questionable, m (>>f): females less attracted than males).

Species	Source	Notch present	Sex attracted
Acanthinus scitulus LeConte	[3]	–	?
Anisotria shooki Young	[3]	–	m (>>f)
Anthicus lutulentus Casey	[3]	–	?
Anthicus nanus LeConte	[3]	–	?
Anthicus punctulatus LeConte	[3]	–	?
Aulacoderus albitarsis van Hille	[2]	m	m
Aulacoderus apterus van Hille	[4]	m	m
Aulacoderus asymmetricus van Hille	[4]	m	m
Aulacoderus bicoloritarsis Pic	[4]	m	m
Aulacoderus bilineatus van Hille	[4]	m	m
Aulacoderus bonadonai van Hille	[5]	m	m
Aulacoderus bradfordi van Hille	[4]	m	m
Aulacoderus brevicornis van Hille	[6]	m	m
Aulacoderus canariensis Wollaston	[4]	m	m (>>f)
Aulacoderus canthariphilus van Hille	[4]	m	m
Aulacoderus cecileae van Hille	[5]	m	m
Aulacoderus colletti van Hille	[4]	m	m
Aulacoderus dorsalis van Hille	[4]	m	m
Aulacoderus flavitarsis Fahr.	[4]	m	m
Aulacoderus flavopictus LaFerte	[4]	m	m
Aulacoderus forchhammeri van Hille	[5]	m	m
Aulacoderus forsythi van Hille	[4]	m	m
Aulacoderus fragilis Fahr.	[4]	m	m
Aulacoderus govenderi van Hille	[4]	m	m
Aulacoderus halleyi van Hille	[7]	m	m
Aulacoderus inopinans Krekich	[4]	m	m
Aulacoderus kochi van Hille	[5]	m	m
Aulacoderus macchleryi van Hille	[4]	m	m
Aulacoderus martini Pic	[4]	m	m
Aulacoderus mediofasciatus Pic	[4]	m	m
Aulacoderus milleri van Hille	[6]	m	m
Aulacoderus mogotoensis van Hille	[4]	m	m
Aulacoderus multidenticulatus van Hille	[4]	m	m
Aulacoderus munroi van Hille	[4]	m	m
Aulacoderus mutatus Gemm.	[4]	m	m
Aulacoderus orangensis van Hille	[4]	m	m
Aulacoderus pedester van Hille	[8]	m	m
Aulacoderus perlucidus van Hille	[8]	m	m
Aulacoderus poweri van Hille	[4]	m	m
Aulacoderus ranchhodi van Hille	[4]	m	m
Aulacoderus recognitus Pic	[4]	m	m
Aulacoderus reverendus Pic	[4]	m	m
Aulacoderus rotundipennis Pic	[8]	m	m
Aulacoderus serowensis Forchhammer	[5]	m	m
Aulacoderus sibayensis van Hille	[8]	m	m
Aulacoderus simoni Pic	[4]	m	m
Aulacoderus smithersi van Hille	[4]	m	m
Aulacoderus techowi Pic	[5]	m	m
Endomia tenuicollis Rossi	[9]	–	?
Formicilla munda LeConte	[3]	–	m, f
Formicomus caeruleus Thunberg	[4]	–	m, f
Formicomus consul LaFerte	[3]	–	m, f
Formicomus gestroi Pic	[25]	–	m, f
Formicomus lacustris Krek.	[8]	–	m, f
Formicomus pedestris Rossi	[1]	–	m, f

Table I: Continued

Species	Source	Notch present	Sex attracted
Formicomus rubricollis LaFerte	[25]	–	m, f
Formicomus tropicalis Krek.	[8]	–	
Hirticomus quadriguttatus Rossi	[9]	–	?
Mecynotarsus balsasensis Werner	[3]	?	?
Mecynotarsus falcatus Chandler	[3]	?	?
Mecynotarsus lacustris van Hille	[8]	m	m
Mecynotarsus obliquemaculatus Marseul	[3]	?	?
Mecynotarsus serricornis Panzer	[9]	?	?
Mecynotarsus vagepictus Fairmaire	[3]	?	?
Microhoria aubei LaFerte	[1]	m	?
Microhoria biauriculatus Pic	[1]	m	?
Microhoria chobauti Pic	[1]	m	?
Microhoria cinctuta Marseul	[1]	m	?
Microhoria fairmairei Brisout	[1]	m	?
Microhoria fasciata Chevrolat	[9]	m	m
Microhoria ghilianii LaFerte	[9]	m	?
Microhoria insignis Lucas	[1]	m	?
Microhoria obscuripes Pic	[10]	?	?
Microhoria terminata Schmidt	[25]	m	m (>>f)
Microhoria tortiscelis Marseul	[3]	m	?
Notoxus allansoni van Hille	[8]	?	m
Notoxus amaculatus van Hille	[8]	m	m
Notoxus anchora Hentz	[11]	m	m
Notoxus bifasciatus LeConte	[3]	?	?
Notoxus brutoni van Hille	[5]	m	m
Notoxus calcaratus Horn	[11]	?	?
Notoxus caudatus Fall	[12]	?	?
Notoxus cavicornis LeConte	[12]	m	m (>>f)
Notoxus celatus Chandler	[11]	?	?
Notoxus conformis LeConte	[12]	m	m
Notoxus cornutus Thunberg	[10]	?	?
Notoxus cucullatus LaFerte	[8]	m	m
Notoxus cumanensis LaFerte	[11]	?	?
Notoxus decorus van Hille	[25]	m	m
Notoxus denutatus Horn	[12]	m	m
Notoxus desertus Casey	[11]	?	?
Notoxus filicornis Casey	[3]	?	?
Notoxus fraternus Champion	[11]	?	?
Notoxus griseofasciatus Pic	[8]	m	m
Notoxus guttulatus Buck	[10]	m	m
Notoxus hageni Chandler	[12]	m	m
Notoxus haustus Chandler	[11]	?	?
Notoxus hiltoni van Hille	[8]	m	m
Notoxus hirsutus Champion	[11]	?	?
Notoxus hirtus LaFerte	[9]	m	?
Notoxus holmi Uhmann	[5]	m	m
Notoxus insitus Quedenfeldt	[13]	?	m
Notoxus intermedius Fall	[3]	m	?
Notoxus lateralis Chandler	[11]	?	?
Notoxus lunulifer Pic	[8]	m	m
Notoxus marginatus LeConte	[11]	?	?
Notoxus mauritanicus LaFerte	[10]	?	?
Notoxus mexicanus Champion	[11]	?	m (>>f)
Notoxus mkuziensis van Hille	[8]	m	m
Notoxus monoceros Linne	[10]	m	m
Notoxus monodon Fabricius	[12]	?	?
Notoxus montanus Casey	[12]	?	?

Table I: Continued

Species	Source	Notch present	Sex attracted
Notoxus murinipennis LeConte	[11]	?	?
Notoxus nevadensis Casey	[12]	m	m
Notoxus nuperus Horn *haustus* Chandler	[12]	?	?
Notoxus ornatus van Hille	[8]	m	m
Notoxus pentheri Pic	[8]	m	m
Notoxus photus Chandler	[3]	?	?
Notoxus pictus Casey	[12]	m	m
Notoxus pygidialis Pic	[3]	?	?
Notoxus reavelli van Hille	[6]	m	m
Notoxus robustus Casey	[12]	m	m
Notoxus roeri Uhmann	[5]	m	m
Notoxus sectator Quedenfeldt	[13]	?	m
Notoxus seminole Chandler	[11]	?	?
Notoxus serratus LeConte	[11]	m	m
Notoxus sparus LeConte	[12]	m	m (>>f)
Notoxus spatulifer Casey	[12]	m	m
Notoxus talpa LaFerte	[11]	?	m (>>f)
Notoxus toltecorum Chandler	[11]	?	?
Notoxus whartoni Chandler	[3]	m	?
Notoxus youngi Chandler	[12]	m	m
Notoxus zapotecorum Chandler	[11]	m	m
Pedilus collaris Say	[3]	?	?
Pedilus elegans Hentz	[3]	?	?
Pedilus impressus Say	[10]	?	?
Pedilus labiatus Say	[10]	?	?
Pedilus lugubris Say	[3]	?	?
Pedilus terminalis Say	[3]	?	?
Pseudoleptaleus unifasciatus Desbr.	[5]	–	m, f
Pseudonotoxus testaceus LaFerte	[5]	–	?
Sapintus javanus Marseul	[3]	–	?
Sapintus malayensis Pic	[1]	?	?
Sapintus plectilis Pic	[3]	–	?
Tenuicomus barnevillei Pic	[9]	m	?
Tenuicomus pumilus Baudi	[1]	m	?
Tomoderus ssp.	[3]	?	?
Vacusus infernus LaFerte	[3]	–	?

Tribe Anthicini of subfamily Anthicinae [14]

The males of four genera of the tribe Anthicini (*Aulacoderus, Clavicomus, Microhoria, Tenuicomus*) of the subfamily Anthicinae possess notches as well. In all species of the genus *Aulacoderus* notches are present [16], and for 43 species an attraction of males to cantharidin is recorded. The genera *Tenuicomus, Clavicomus* and *Microhoria* include species that lack elytral notches in the males. It is not known if species without notches are also canthariphilous. Two species of *Tenuicomus* are reported to be canthariphilous and both species have elytral notches. Also all 21 species of this genus in the collection of Uhmann (personal communication) have these organs. Some species of the genus *Clavicomus* possess elytral notches but no species is recorded to be attracted to cantharidin. Eleven species of the genus *Microhoria* show an attraction to cantharidin in the field. Ten species of this genus had elytral notches, but for one species no literature data were available. According to Table I, all species of the discussed genera of the tribe Anthicini that were mainly baited with cantharidin were males, but some females were attracted in a number of species as well.

Species of the genus *Tomoderus* seem to possess notch-like elytral structures as well. At the apex of the male elytron of *Tomoderus besuchetianus* [17] lies a little swelling and nearby is a slight depression. Whether this structure is analogous to the elytral notches of above listed genera has still to be determined.

From none of the listed canthariphilous species of the genera *Acanthinus* (1 species), *Anthicus* (3 species), *Endomia* (1 species), *Formicilla* (1 species), *Formicomus* (7 species), *Hirticomus* (1 species), *Pseudoleptaleus* (1 species), *Sapintus* (3 species) and *Vacusus* (1 species) was an elytral notch recorded. Both males and females are attracted to cantharidin in the genera *Formicomus* and *Pseudoleptaleus*.

Tribe Pedilini of subfamily Pedilinae [14]

Of the subfamily Pedilinae six species of *Pedilus* are recorded as being canthariphilous [18, 3]. Both sexes in that genus are said to have simple elytral notches [18]. Our investigations could not confirm the presence of notches, at least for the species *P. fuscus* [18]. Both sexes of this species showed totally homogeneous elytral apices devoid of notches. Mainly males are attracted to cantharidin in the species *Anisotria shooki* but no notches are recorded. Females of this species have reduced wings [19].

Morphology of the elytral notch of Notoxus monoceros as indicated by scanning electron microscopy

The elytral notches of male *Notoxus monoceros* were examined in detail (Fig. 1 and 2). At the outer rim of the apex of each elytron there is an oval depression which is mostly surrounded by a tuft of hairs (Fig. 2A, B). At the bottom of the distinctly sclerotized cavity, cuticular cones and small pores are evident from outside. Outer and inner membranes of the elytra do not stick together apically, and they form a reservoir-like space (Fig. 2C, E). A cross-section of this area shows several trabecular-like structures, extending from the outer to the inner elytral membrane (Fig. 2E). In the KOH-macerated elytral apex removal of the soft inner elytral membrane reveals an internal cone, which is connected with numerous glandular tubules (Fig. 2F). The tips of the tubules are enlarged apically into chitinous bulbs (Fig. 2G). In fresh

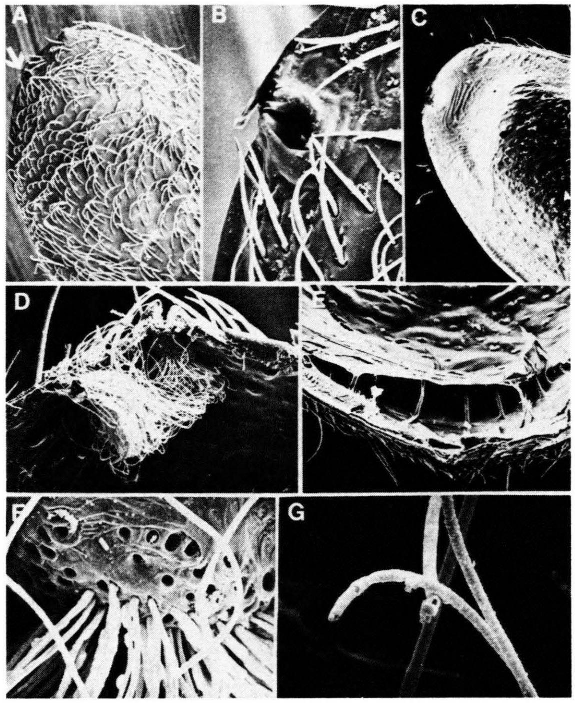

Fig. 2. Elytral notch of a male *Notoxus monoceros* (SEM). A: outer view of notch at the tip of the elytron (arrow, 133 ×), B: elytral notch (842 ×), C: inner view of the elytron with apical swelling (90 ×), D: glandular tubules of notch leading to a cuticular cone (macerated, 454 ×), E: cross-section of the apical elytral swelling; trabecular-like structures extent from the inner to the outer elytral membrane (243 ×); F: cuticular cone with glandular tubules (macerated, 2850 ×); G: glandular tubule ending into a chitinous bulb (macerated, 5450 ×).

preparations of elytral apices, the gland cells of the notch form a bilobed gland (Fig. 1) which is packed within this peculiar elytral excavation.

Quantification of cantharidin in elytral notches and other external and internal structures of male Notoxus monoceros

Cantharidin-fed beetles in the laboratory

Male field-caught *Notoxus monoceros* readily devour offered cantharidin crystals. All individually analyzed notches of 12 *Notoxus monoceros* specimens previously fed with cantharidin contained this monoterpene (Fig. 3). The mean cantharidin titre ($\bar{x}$) within the notches of the twelve fed beetles was 1.72 ng cantharidin/μg body weight (w). The mean value rises from 1.72 ng to

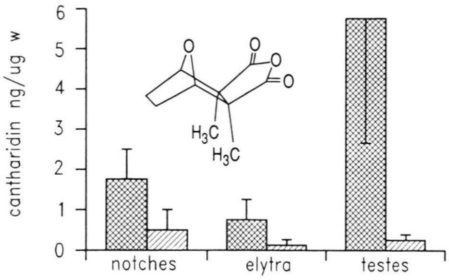

Fig. 3. Means of cantharidin-titres (cantharidin ng/µg body fresh weight) in notches, elytra and testes of 12 specimens of *Notoxus monoceros* fed with cantharidin-crystals for two (2 individuals), three (2 individulas) and four days (8 individuals) (cross-hatched bars) and 12 unfed field caught specimens (hatched bars); bars with corresponding standard deviations.

2.0 ng cantharidin/µg w and the standard deviation decreases from 0.76 to 0.72 only within those individuals that were fed cantharidin during four days. The mean cantharidin-titre of the four beetle specimens fed two and three days on cantharidin is clearly lower (1.12 ng/µg w). The average value of cantharidin of other elytral fragments of cantharidin-fed individuals (one fragment per individual) is high significantly lower (0.81 ng/µg w;**) compared with notches. The testes (one testicle per individual) of cantharidin-fed individuals contain very high amounts of the monoterpene ($\bar{x} = 5.82$ ng/µg w, $s = 3.17$). The maximum value may even reach 11.5 ng cantharidin/µg w in one notch of a specimen. Considering only those eight beetles that ingested cantharidin during 4 days, the mean value of the male internal organs rises to 7.4 ng/µg bodyweight ($s = 0.73$). The significance for the 12 males of *Notoxus monoceros* between cantharidin-titre of the testes is very high in comparison with the notches***, as well as with the elytral fragments***. In two cantharidin-fed specimens flight muscles and middle legs were analyzed. The average value for the legs is 0.47 ng cantharidin/µg bodyweight and 0.1 ng/µg w in the muscles. These titres of the other body parts are therefore distinctly lower as compared with elytral fragments (0.81 ng cantharidin/µg w), notches (1.72 ng cantharidin/µg w), and testes (5.82 ng cantharidin/µg w).

** highly significant; *** very highly significant; see Methods and Materials.

Field caught *Notoxus monoceros* with no feeding-access to cantharidin

The elytral notches (two per individual) of unfed field-caught individuals contained on average 0.45 ng cantharidin/µg w ($s = 0.43$; Fig. 3). The titres of specimens from southeastern (Bayreuth) and southwestern (Karlsruhe) Germany showed interesting differences. From the three specimens trapped near Bayreuth only one notch contained minute amounts of cantharidin (0.3 ng/µg w). The maximum value was in a southeastern specimen from Karlsruhe with 1.4 ng/µg w per notch. In four individuals of the 12 investigated specimens cantharidin was completely absent.

The average value of other elytral parts (one fragment per individual) from unfed specimens amounts 0.05 ng/µg w. Only one beetle from Karlsruhe reached 0.56 ng cantharidin/µg w. Eleven specimens contained no cantharidin at all. Differences of means between notches and elytral parts were highly significant**.

Very small amounts of cantharidin (0.12 ng/µg w, $s = 0.26$) were also present in the testes (one testicle per individual) of unfed individuals. Only three specimens from Karlsruhe proved to have cantharidin within their testes at all.

The mean cantharidin value of the notches of unfed beetles is highly significantly different from values of remaining elytral parts** and significantly different from the testes*. There is no significant difference between means of testes and elytral parts of the unfed specimens.

Generally beetle specimens from Karlsruhe exhibited elevated cantharidin titres in all body parts such compared with beetles from Bayreuth localities.

A comparison between means of cantharidin-titres of notches, elytral fragments and testes of fed and unfed beetles showed in all cases significance at a 0.001% confidence level***.

To make a comparison of the cantharidin-titres of the fresh weights of elytral parts and notches with the dry weights of the testes, the dry weights of elytral parts and notches were determined for five specimens. The difference between fresh and dry weights for the elytral structures was only 5–10%. Hence fresh and dry weights are nearly identical. On the other hand the loss of weight for the testes was 95–90% from the fresh weights. Therefore, fresh and dry weights of the testes differ enormously. Thus, the established cantharidin-titres of the dry weights of testes and fresh weights of the elytral structures are comparable.

Discussion

A small fraction of anthicid beetles are characterized by the presence of the elytral notches in the males. Cantharidin seems to have the same function in all species: apparently only male individuals are attracted to it, but some females of most species can be trapped as well on this bait [12]. The results of the investigations of the elytral notches of *Notoxus monoceros* lead to the assumption that an analogous cycle as described by Eisner 1988 [20] in *Pyrochroa flabellata* exists in anthicid species with elytral notches. Male individuals of *Pyrochroa flabellata* are attracted by cantharidin and ingest offered crystals. During copulation the terpene is transfered to the female, which transmits the insecticidal substance into its eggs. Eggs and larvae seem thus protected from predators. Central in the sexual behaviour of that species is a glandular structure on the head of the male. After an intake of cantharidin this substance can be found in this impression or cavity. Females might thus be able, prior to copulation, to test how much cantharidin the partner is able to invest in their common breed. A male without cantharidin in the head cavity is not accepted by the female [20].

The chemical analyses of cantharidin-fed male specimens of *Notoxus monoceros* show a considerable accumulation of cantharidin in the elytral notches (1.2–3.5 ng/μg w) and in the testes (6.3–11.5 μg/μg w; Fig. 3). It is obvious that only a certain quantity of cantharidin is secreted into the notches, whereas the quantity in the testes seems to depend on the total intake. Notches therefore have a lower capacity for cantharidin storage than the testes. Analyses of other organs of the beetles indicate no further store places of cantharidin in the body. Thus, there are two main reservoirs for cantharidin in the body of the beetle, notches and testes. Notches seem to function as a close-range "test-organ" for the females, while the testes seem to transmit the accumulated cantharidin from the male to the female during copulation. In the few couples of *N. monoceros* available for study, laboratory observations of sexual display showed that the female actually bites several times into the notches of cantharidin-fed males before a copulation takes place. Unfed males were obviously not accepted. Thus a sexual selection is effected by the females. The important role of notches in the sexual behaviour of *Notoxus monoceros* is further underlined by the fact that beetles without access to cantharidin concentrate most of the monoterpene they collected from natural resources (*e.g.* meloid beetles) into their notches (Fig. 3). Even small amounts of cantharidin accumulated in this peculiar elytral structure could therefore be enough for a successful close-range-courtship behaviour and a subsequent copulation. Thus, cantharidin presentation is an advantage for the male in the competition for a female partner.

The morphological results suggest a function of the elytral notch organ as a kidney-like structure that filters cantharidin out of the haemolymph. The whole bilobed gland, formed by single gland cells with long ductules, lies in the haemolymph of the elytral apex reservoir. Ingested cantharidin is probably actively transported from the intestines into the haemolymph of the insect. This is demonstrated by the low cantharidin-titre ($x = 0.3$ ng/μg w) in different parts of the body (muscles, legs, elytral parts). From the haemolymph, free or protein bond cantharidin is accumulated by gland cells of the elytral organ und by testes. The results in unfed specimens of *Notoxus monoceros* indicate a specific time-dependent accumulation where first the notches are "filled" with cantharidin before the surplus is transferred to the tests for storage. Analogous close-range "test-organs" can be found in glands of the head cavities of males of european Pyrochroidae species *Pyrochroa coccinea* and *Schizotus pectinicornis* [25].

While cantharidin seems to function as a long-range pheromone for the males, the same compound exhibits only a close-range activity for females. If there is an equal sex-ratio in the field, the small number of attracted females could be due to:

– their reduced perceptive capacity for cantharidin. If cantharidin functions as a sexual pheromone for females, a perception in the close surroundings during sexual display is sufficient, while males must be perceptive over longer distances. Increased cantharidin sensitivity might therefore be of advantage for the males to collect more cantharidin and thus increase the chances of a successful reproduction.

– reduced wings of the females of a number of *Notoxus*-species. The females are not able to fly, while the males are good flyers [15]. This could as well be a reason for the low number of attracted females of some species.

– a male long-range pheromone. Males have been observed to evert abdominal gland vesicles in the presence of females [25].

A comparison of beetles that fed for 4 days on cantharidin with specimens fed for two and three days shows that the process of cantharidin accumulation into notches and testes obviously takes several days. The concentration of cantharidin in elytral parts of beetles fed for 2 and 3 days on cantharidin crystals is relatively high, whereas the titre in notches and testes is lower compared with beetles fed for 4 days on cantharidin.

Interesting differences in cantharidin-titres are obvious between field caught specimens from southwestern (Karlsruhe) and southeastern (Bayreuth) Germany. The 3 specimens from Bayreuth contained no cantharidin except for a minor amount in one notch of an individual. Only in 2 specimens from Karlsruhe was no cantharidin registered, while 7 males showed some cantharidin at least in the notches (3 individuals). One southwestern beetle specimen contained nearly as much cantharidin in the notches as was found in cantharidin-fed individuals. This observation leads to known natural cantharidin sources (meloid and oedemerid beetles) and their availability in the field. Assumptions concerning the existence of other natural sources of cantharidin are based on the notion that such a complex system as formed by the canthariphilous insects must be based on a better availability of cantharidin. Thus, analogous substances in plants or fungi or other natural sources of cantharidin are being discussed [21]. The differences in cantharidin-titres between specimens from Bayreuth and Karlsruhe, however, indicate that the conventional sources of meloid and oedemerid beetles may be the only sources. Meloid beetles (with high cantharidin-titres in the haemolymph [22]) are absent in the surroundings of Bayreuth, which has a more continental climate, and even oedemerid beetles (low cantharidin-titres [23]) are rare here. In Karlsruhe, situated in the rift valley of the river Rhine with yearly higher temperature means, meloid and oedemerid beetles are much more common and hence the possibility for an intake of cantharidin [24] increases considerably. This difference in the availability of natural cantharidin sources seems to be reflected in the cantharidin-titres of the analyzed males of *Notoxus monoceros* from Bayreuth and Karlsruhe.

Acknowledgements

Mr. G. Uhmann (Pressath) kindly confirmed identity of captured anthicid beetles and contributed highly valuable data from his Anthicidae collection. Many thanks are due to an anonymous reviewer for valuable contributions and to Prof. Dr. L. Newton (Nairobi) for improving the English version of the manuscript.

[1] K. Görnitz, Arb. phys. angew. Ent. Berlin-Dahlem **4,** 116–157 (1937).
[2] J. C. van Hille, S. Afr. J. Sc. **51,** 154–155 (1954).
[3] D. K. Young, Great Lakes Entomol. **17,** 187–194 (1984).
[4] J. C. van Hille, Ann. Cape Prov. Mus. **15** (1984).
[5] P. Forchhammer, Botswana Notes Rec. **17,** 163–174 (1985).
[6] J. C. van Hille, Durban Mus. Nov. **18,** 155–168 (1984).
[7] J. C. van Hille, Ann. Cape Prov. Mus. **16,** 321–326 (1988).
[8] J. C. van Hille, Trans. R. Soc. S. Afr. **39,** 367–391 (1970).
[9] M. A. Bologna, Boll. Assoc. Rom. Entomol. **39,** 77–82 (1984).
[10] M. Abdullah, Ann. Mag. nat. Hist. **7,** 247–254 (1964).
[11] D. S. Chandler, Contr. Am. Entomol. Inst. **15,** 1–83 (1977).
[12] D. S. Chandler, Entomography **1,** 33–438 (1982).
[13] F. Karsch, Entomol. Nachr., 51–54 (1885).
[14] G. Uhmann, Entomol. Blätter **74,** 75–80 (1978).
[15] J. C. van Hille, in: South Afr. animal life, chapter 3, 217–258 (1961).
[16] J. C. van Hille, Ann. Cape Prov. Mus. **15** (1984).
[17] G. Uhmann, Rev. Suisse Zool. **4,** 687–701 (1987).
[18] M. Abdullah, Dtsch. Entomol. Z. **16,** 323–366 (1969).
[19] D. K. Young, Coleop. Bull. **38** (2), 201–208 (1984).
[20] T. Eisner, Verh. Dtsch. Zool. Ges. **81,** 9–17 (1988).
[21] M. Frenzel, K. Dettner, D. Wirth, J. Waibl, W. Boland, Experientia **48,** 106–111 (1992).
[22] J. P. McCormick, J. E. Carrel, chapter 10, in: Pheromone Biochemistry (eds. G. D. Prestwich, G. J. Blomquist), 307–350, Academic press, Orlando 1987.
[23] J. E. Carrel, J. P. Doom, J. P. McCormick, J. Chromatogr. **342,** 411–415 (1985).
[24] P. Havelka, Veröff. Naturschutz Landschaftspflege Bad.-Württ. **57/58,** 181–202 (1984).
[25] Personal observations.