Oecologia (1994) 100:421–429
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ORIGINAL PAPER

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Evaluation of the palatability of chrysomelid larvae containing anthraquinones to birds

Received: 18 July 1994 / Accepted: 15 September 1994

Abstract Chrysomelid larvae of the subfamily Galerucinae, tribe Galerucini, are known to contain 1,8-dihydroxylated 9,10-anthraquinones. Since nonhydroxylated 9,10-anthraquinone is the active agent in several commercial products sold to protect seeds against birds, we suggested that the naturally occurring dihydroxylated anthraquinones of galerucine larvae may also act as protective devices against bird predation. Tits (*Parus* spp.) are potential predators of larvae of the tansy leaf beetle, *Galeruca tanaceti*, and the elm leaf beetle, *Xanthogaleruca luteola*. To investigate the palatability of these chrysomelid larvae to birds, we offered them with mealworms and *Calliphora* pupae, respectively, as controls in dual choice bioassays to eight singly kept, naive tits (five *P. major* and three *P. ater* individuals). The bioassays were limited to 5 days, during which larvae were offered daily for 2 h (*X. luteola*) and 3 h (*G. tanaceti*), respectively. Every day, the birds significantly avoided uptake of *G. tanaceti* and *X. luteola*. More than 98% of the control food was consumed daily, whereas the percentage of chrysomelid larvae totally eaten never surpassed 6.6% for *G. tanaceti* and 51.8% for *X. luteola*. In order to determine whether this avoidance was due to the anthraquinones of the chrysomelid larvae, mealworms and *Calliphora* pupae, respectively, were treated with these compounds in concentrations equivalent to the natural ones. Dual choice bioassays with treated and untreated prey were conducted, again for 5 days with a daily 2- or 3-h test period, respectively. The tits ate all or nearly all treated and untreated food items every day. However, during the 5-day test period the tits learnt to take up the control insects significantly earlier than the treated ones; the food containing anthraquinones was not consumed as readily as the control, which suggests aversive learning based on distastefulness. The efficiency of anthraquinones in protecting galerucine larvae against bird predation is discussed with special respect to learning behavior and factors which might delay or mask learning of avoidance.

Key words Chrysomelidae · Galerucinae
Anthraquinones · Avian deterrent · Unpalatability

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Introduction

Chemical protection of insects against predation by birds has been demonstrated in numerous studies (e.g. Coppinger 1969; Bowers and Farley 1990; Evans and Schmidt 1990; Mason et al. 1991; Marples 1993). A famous example is the protection of the monarch butterfly *Danaus plexippus* against the blue jay (*Cyanocitta cristata*), which vomits this lepidopteran prey because of its bitter tasting cardenolides (Brower et al. 1988 and references therein).

A commonly used deterrent against birds is 9,10-anthraquinone (Fig. 1 A). This substance is commercially applied in orchards to protect buds against damage e.g. by the bullfinch *Pyrrhula pyrrhula* (Meier 1978; Zbinden 1976). It is also used for protection of seeds against crows (*Corvus* spp.). This anthraquinone is known to be naturally present only in the cuticular wax of *Lolium perenne* (Gramineae) and in the essential oil of *Nicotiana tabacum* (Solanaceae) (Thomson 1987). While the natural occurrence of nonhydroxylated anthraquinone is rare, 9,10-anthraquinones hydroxylated in positions 1,2 and 1,8, respectively, are widespread within the plant kingdom and scattered over various animal taxa such as Polychaeta, Asteroidea, Crinoidea, Coccoidea and Coleoptera (Goodwin 1971; Kayser 1985; Thomson 1987). Within the Coleoptera, the occurrence of anthraquinones is – as far as known – restricted to the tribe Galerucini of the chrysomelid subfamily Galerucinae. The anthraqui-

nones chrysazin, chrysophanol and the anthrone dithranol have been identified from eggs, larvae and adults of several of these coleopteran species (Hilker and Schulz 1991; Hilker et al. 1992; Howard et al. 1982; Fig. 1 B–D). The close structural relationship between these galerucine anthraquinones and 9,10-anthraquinone suggests that the leaf beetles' anthraquinones might serve for protection against avian predators. In order to investigate the role of anthraquinones for protection against birds, we tested whether larvae of the tansy leaf beetle *Galeruca tanaceti* and the elm leaf beetle *Xanthogaleruca luteola* are palatable to tits (*Parus* spp.), which are potential predators often present in habitats of these galerucines. The following questions were addressed in four experiments (Table 1):

1. Do tits attack and consume larvae of *G. tanaceti*, which are black and possess many setae? Do they learn to avoid uptake of these larvae during a test period of 5 days? (experiment 1).
2. Is the tits' avoidance of *G. tanaceti* larvae due to the anthraquinones? (experiment 2). The same questions were examined in *X. luteola* larvae, which are coloured dark-yellowish and have only few bristles (experiments 3 and 4).

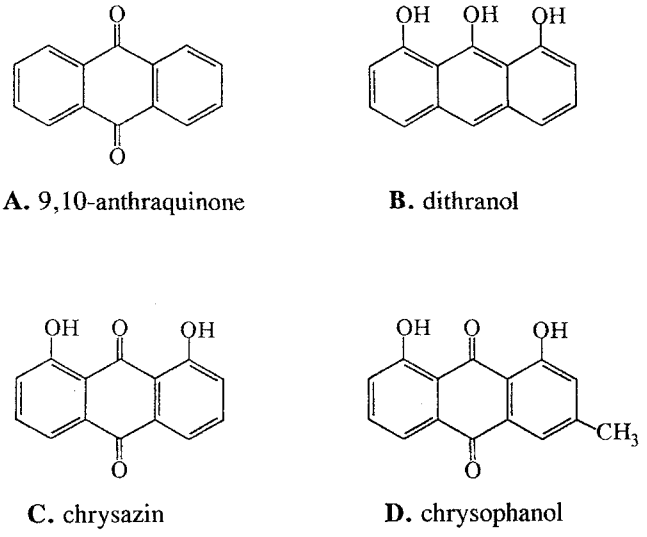

Fig. 1 **A** 9,10-Anthraquinone known as avian repellent. **B**, **C** and **D** anthrone and anthraquinones known from Galerucinae

Material and methods

Birds

Experiments were carried out with five *P. major* and three *P. ater* individuals, which were taken out of their nests at the age of 12–18 days and transferred to an artificial nest box, where they were fed with drone brood (*Apis mellifica*) offered with forceps. After about a week in this artificial nest, the birds were set separately into cages of 60×120×120 cm size each. The cages were placed outdoors on the flat roof of our laboratory. At the age of about 4 weeks, all tits were able to take up the drone brood on their own from petri dishes (9 cm diameter) placed on the bottom of each cage. At the age of about 5 weeks, a food mixture of ground beef heart, chopped dried insects, seeds and water was offered to the birds instead of drone brood. Experiment 1 started when the birds were 5–6 weeks old. Experiments 2, 3, and 4 were started 1 week after the beginning of the preceding experiment (Table 1).

Insects

Larvae of the tansy leaf beetle *G. tanaceti* were collected in the field near Bayreuth, kept in the laboratory on *Achillea millefolium* until the third instar, and then were stored frozen at –40°C.

Larvae (L3) of the elm leaf beetle *X. luteola* were collected in southern France and also kept frozen at –40°C.

Laboratory reared, final instar larvae of *Tenebrio molitor* and pupae of *Calliphora* sp., respectively, were frozen on dried ice and used as controls (see Table 1).

Experiments

Each experiment lasted 5 days, during which larvae containing anthraquinones and untreated control mealworms or *Calliphora* pupae were offered simultaneously in separate petri dishes, placed next to each other on the bottom of a cage. Two observers registered the time when a bird pecked, partly ate, or consumed (completely swallowed) the prey. Table 1 shows what was offered to every bird per day in each experiment. Each experimental day started at 8 a.m., and the daily experimental period lasted 3 h in experiments 1 and 2, and 2 h in experiments 3 and 4. During the experiments the birds were fed at noon and 5 p.m. with the food mixture described above. Prior to the experiments we determined the average daily consumption of this food for each bird. This ranged from 19 to 25 g in *P. major* and from 16 to 17 g in *P. ater*.

Table 1 Experimental designs: each experiment lasted 5 days (*AQ* anthroquinone)

Experiment	Daily diet		Duration of daily experimental period	Specials
	Test	Control		
1	15 *Galeruca tanaceti* larvae	15 untreated mealworms	180 min	none
2	10 AQ treated mealworms (conc.=*G. tanaceti* larvae)	10 mealworms treated with solvent	180 min	control petri dish marked with a red stripe; AQ-treatment: topical
3	7 *Xanthogaleruca luteola* larvae	7 untreated *Calliphora* pupae	120 min	none
4	7 AQ treated *Calliphora* pupae [a]	7 *Calliphora* pupae treated with solvent	120 min	control petri dish marked with a red stripe; AQ-treatment: injection

[a] Concentration as in *Pyrrhalta viburni* larvae

The same amount of food was offered to the birds during the 5-day test period, in order to standardize their level of hunger at the beginning of each experimental day.

In experiments 1 and 3, larvae of *G. tanaceti* and *X. luteola*, respectively, were offered in order to investigate whether the naive birds, which had no prior experience of coleopteran larvae, could distinguish between these chrysomelid larvae and the simultaneously offered controls. Experiments 2 and 4 were conducted in order to examine whether the avoidance of *G. tanaceti* and *X. luteola* – observed in experiments 1 and 3 – is due to the anthrones and anthraquinones of these larvae.

Quantitative GC-MS analyses revealed that a single *G. tanaceti* larva (L3) contains 28.72 ± 0.929 µg chrysophanol (0.06% of the body weight) and 19.35 ± 2.839 µg chrysazin (0.04% of the body weight) (method see Hilker 1992). Each test mealworm (referred to below as AQ-mealworm, AQ-larvae, AQ-prey, or AQ-food) was treated with anthraquinone concentrations equivalent to those found in *G. tanaceti* larvae. For application of chrysophanol and chrysazin, these compounds were dissolved in dichloromethane. The solution was slowly dropped onto the mealworm, while evaporating the solvent with a hair-dryer. Application of the solvent onto the control mealworms was conducted in the same manner.

In experiment 4, a dichloromethane solution of chrysophanol, chrysazin and dithranol was injected into *Calliphora* pupae (referred to below as AQ-pupae, AQ-prey, or AQ-food). The number of *X. luteola* larvae collected was insufficient for quantitative determination of the anthraquinone/anthrone content. Thus, a dichloromethane solution of these compounds was used in concentrations equivalent to the known concentrations of chrysophanol, chrysazin, and dithranol in *Pyrrhalta viburni* larvae: 11.85 ± 0.779 µg chrysophanol (0.09% of the body weight), 15.84 ± 1.021 µg chrysazin (0.12% of the body weight), and 7.74 ± 1.224 µg dithranol (0.06% of the body weight) (Hilker 1992). *X. luteola* larvae look quite similar to *P. viburni* larvae. Kimoto (1989) even assigned *X. luteola* to the genus *Pyrrhalta*, whereas Kippenberg (1994) argues for keeping it within the genus *Xanthogaleruca*. Injections of the anthraquinone solution into the test pupae and of dichloromethane into the controls were conducted 24 h prior to the experiments, so that the solvent could evaporate at room temperature.

In experiments 2 and 4, the test and control food could not be distinguished optically. Furthermore, the tits knew that untreated mealworms and pupae were palatable from the previous experiments 1 and 3. In order to give the birds the chance to learn to distinguish between treated and untreated food, the petri dishes in which the control prey was offered were marked with a red stripe in the middle. Behavioral observations prior to the experiments revealed that the tits at first always avoided uptake of unknown food or food offered in dishes they were not familiar with. Thus, with this optical marking of the control dishes we accepted that the birds at first might avoid the palatable control food. Nevertheless, we hypothesized that during an experimental time of 5 days they would have time to learn to connect unpalatability with the food offered in unknown petri dishes. If the petri dishes with the anthraquinone-treated food had been marked and if birds had avoided uptake of this food, the results would not have provided clear information on whether avoidance behavior was due to the anthraquinones or to the marking.

Statistical analyses

Data were statistically analyzed by the Wilcoxon matched-pairs signed-rank test and by repeated-measures ANOVAs.
The ANOVAs addressed the following questions:

1. Did uptake of food (test and control insects) differ between the individual birds within the 5-day test period (factor "birds")?
2. Did uptake of test and control insects differ from each other within the 5-day test period? (factor "food")?
3. Did uptake of food (test and control insects) differ between the days of a test period, when considering feeding of all birds together (factor "day")?
4. Was there an interaction between the uptake of food for individual birds and the experimental day (factor "day×bird")?
5. Was there an interaction between the uptake of test vs. control insects and the experimental day (factor "day×food")?

The variable "uptake of food" includes pecked, partially eaten, or ingested insects (= prey uptake). It is the integral of the percentage of test and control insects taken up over time. Analyses of variance examine uptake of food during the first 90 min for experiments 1 and 2, and during the first 30 min for experiments 3 and 4. After these time intervals, more than three-quarters of the control insects had been consumed, so that the choice situation was no longer favourable. The repeated-measures ANOVAs do not assume independence of the dependent variables (SAS 1988).

Using the Wilcoxon test, we compared the uptake of test and control insects by the eight birds (= number of replications). These comparisons were made for predetermined distinct time intervals of the 2-h and 3-h test periods, respectively, of each experimental day. We did not observe any differences in feeding behavior between *P. major* and *P. ater* when the test insects were offered. Thus, data obtained from observations of both species of birds were pooled.

Results

Experiments 1 and 3 revealed that the tits avoided *G. tanaceti* and *X. luteola* larvae (Table 2).

In experiment 1, the tits daily ate all or nearly all control mealworms; very few mealworms remained untouched on day 1 and 5. On the other hand, larvae of *G. tanaceti* were rarely ingested during the 1st day. The percentage of completely eaten *G. tanaceti* larvae was highest during the 2nd day (6.6%), but decreased again to 2.5% on the 5th day. The number of *G. tanaceti* larvae that were not pecked at all was highest during the 1st day (75.8%), but decreased to 35.0% on the 2nd day. It increased again to 62.5% on the 5th day. Examples of *G. tanaceti* larvae that were just pecked or only partly eaten are shown in Fig. 2.

Table 2 Behavioural responses of tits towards test and control insects offered during a test period of 3 h in experiment 1 and of 2 h in experiment 3. Test insects: *G. tanaceti* (n=120; 15 for each of the 8 tits) and *X. luteola* (n=56; 7 for each of the 8 tits) larvae, respectively. Control insects: mealworm (n=120; 15 for each of the 8 tits) and *Calliphora* pupae (n=56; 7 for each of the 8 tits), respectively

Day	% Controls eaten	% Larvae eaten	% Larvae eaten partially	% Larvae pecked	% Larvae untouched
Experiment 1: *G. tanaceti* larvae vs. control mealworms					
1	98.3	0.8	1.7	21.7	75.8
2	100	6.6	39.2	19.2	35.0
3	100	1.7	29.2	22.5	46.6
4	100	2.5	45.8	10.0	41.7
5	99.2	2.5	31.7	3.3	62.5
Experiment 3: *X. luteola* larvae vs. control *Calliphora* pupae					
1	100	25.0	28.6	26.8	19.6
2	100	51.8	26.8	12.5	8.9
3	100	17.8	30.4	25.0	26.8
4	100	33.9	35.7	16.1	14.3
5	100	7.1	32.1	32.2	28.6

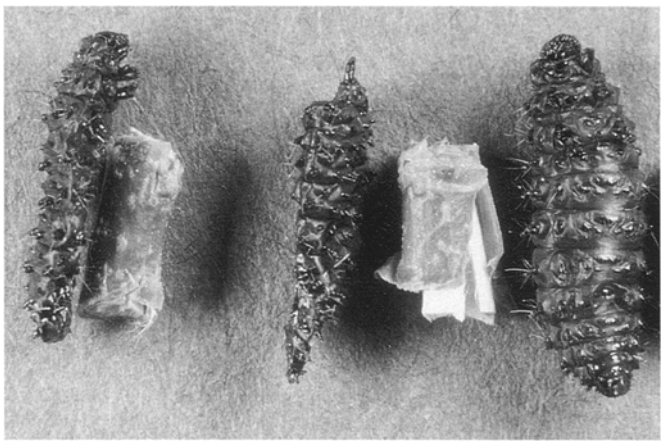

Fig. 2 Larvae of *Galeruca tanaceti*. *Left* having been pecked by a tit; *middle* having been partly eaten by a tit; *right* intact larva. Between the larvae are pieces of a plant stalk

While less than 1% of the *G. tanaceti* larvae offered were eaten during the 1st day of experiment 1, a quarter of the *X. luteola* larvae were ingested during the 1st day of experiment 3. As in the experiment with *G. tanaceti* larvae, the proportion of *X. luteola* larvae eaten was highest during the 2nd test day: more than half of them were totally consumed during this day. However, on the 5th day the percentage of *X. luteola* larvae eaten declined to 7.1%. The percentages of partially eaten *X. luteola* larvae ranged from 26.8% to 35.7%, and from 12.5% to 32.2% for pecked larvae.

Uptake of food significantly changed in the course of time during the 5-day test periods of experiments 1 and 3 (Table 3, factor "day"). When looking in detail at the temporal uptake of test and control food during each experiment day, the results of experiments 1 and 3 showed that the rate of uptake (i.e. pecked, partly eaten, totally eaten insects) of the chrysomelid larvae increased at about the time when more than 80% of the controls had been eaten.

In experiment 1 (Fig. 3A), the uptake of mealworms was quite slow during the first minutes of the 1st day, but then increased rapidly. The following days, mealworms were readily eaten as soon as they were offered to the tits. On the first day, the uptake of *G. tanaceti* larvae increased after 2 h, whereas on days 2 and 3 uptake of these larvae started to rise 60–90 min after the test started. During days 4 and 5, the situation remained very similar to that on day 3. During the 5-day test period, the uptake of *G. tanaceti* larvae was significantly lower than that of the mealworms (Table 3, factor "food"). The rates of uptake of test and control prey were statistically compared for the following distinct time intervals of the daily 3-h test period: 0–10, 0–20, 0–30, 0–60, 0–90, 0–120, 0–150, and 0–180 min. The results of these comparisons are summarized in Table 4 and illustrate differences between uptake of test and control insects as shown in Figs. 3 and 4. Significantly more mealworms than *G. tanaceti* larvae were taken up

Table 3 Repeated-measures ANOVAs of variable "uptake of food" by 8 tits during 5 experimental days for experiment 1–4

Source	*df*	MS	*F*	*P>F*
Experiment 1: *G. tanaceti* larvae vs. control mealworms				
Between-subject factors				
Bird	7	73661.0	2.5	n.s.
Food	1	15395737.8	527.1	0.0001
Error	7	29207.1		
Within-subject factors				
Day	4	140148.1	10.9	0.0001
Day×bird	28	10658.1	0.8	n.s.
Day×food	1	62458.1	4.8	0.0042
Error	28	12855.9		
Experiment 2: AQ mealworms vs. control mealworms				
Between-subject factors				
Bird	7	201263.8	8.8	0.0050
Food	1	167902.8	7.3	0.0298
Error	7	22707.2		
Within-subject factors				
Day	4	28214.0	1.3	n.s.
Day×bird	28	5448.4	0.2	n.s.
Day×food	4	104284.8	4.9	0.0036
Error	28	20893.9		
Experiment 3: *X. luteola* larvae vs. *Calliphora* pupae				
Between-subject factors				
Bird	7	675.1	0.5	n.s.
Food	1	357781.2	287.7	0.0001
Error	7	1243.5		
Within-subject factors				
Day	4	111692.2	23.9	0.0001
Day×bird	28	664.3	1.4	n.s.
Day×food	4	2056.3	4.4	0.0069
Error	28	467.2		
Experiment 4: AQ *Calliphora* pupae vs. control *Calliphora* pupae				
Between-subject factors				
Bird	7	7140.8	3.03	n.s.
Food	1	16979.8	7.21	0.0313
Error	7	2355.2		
Within-subject factors				
Day	4	1618.4	3.27	0.0254
Day×Bird	28	539.1	1.09	n.s.
Day×Food	4	398.4	0.81	n.s.
Error	28	494.5		

at each time interval of each day except day 1. During this 1st day, there was no significant difference between uptake of *G. tanaceti* larvae and mealworms during the first 30 min.

In experiment 3 (Fig. 4A), the temporal analyses of the uptake of *X. luteola* larvae and control pupae revealed that, as in experiment 1, the uptake of control insects was quite slow during the 1st experimental day, but increased rapidly during the following days. From the 1st day on, the tits took up *X. luteola* larvae later than the *Calliphora* pupae. During days 4 and 5, the sit-

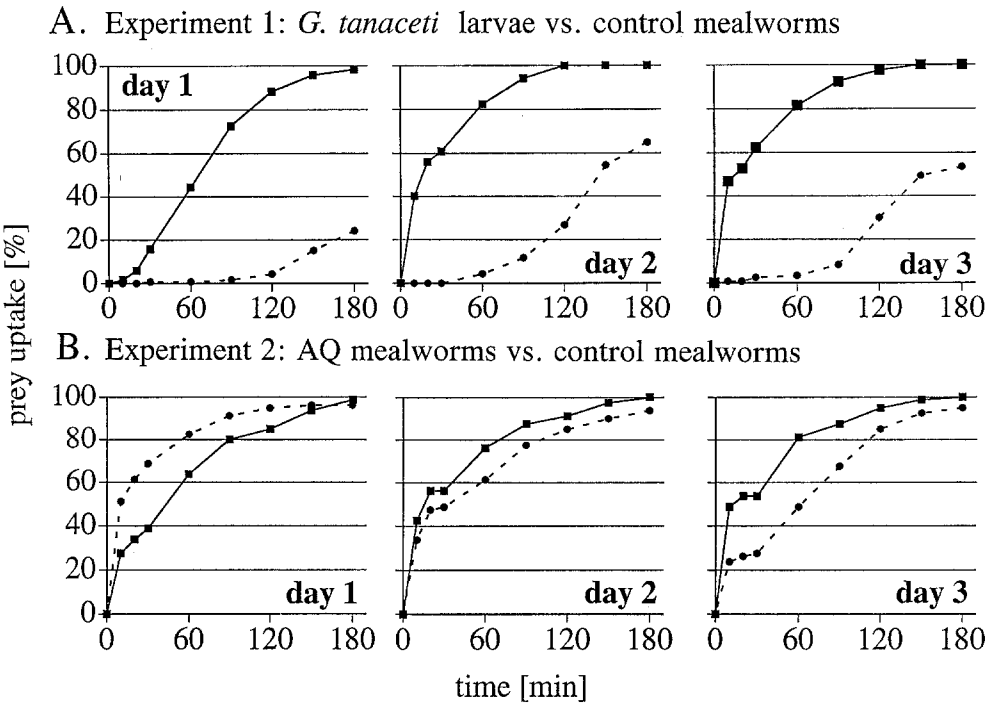

Fig. 3 Uptake of prey offered in experiments **A** 1 and **B** 2 during the 3-h test periods of days 1–3. The figures show the percentages of control mealworms (*solid lines*), *Galeruca tanaceti* larvae and anthraquinone (*AQ*)-treated mealworms (both *dashed lines*) taken up by the 8 tits. Prey uptake by the 8 tits was summarized. The term "prey uptake" includes pecked, partially eaten, and ingested insects. Further details of experiments 1 and 2 are given in the text

Table 4 Statistical evaluation of experiments 1–4 by the Wilcoxon matched-pairs signed-rank test. Levels of significances of differences between uptake of test and control insects during distinct time intervals of day 1, 2, and 3. Each time interval given begins at test start (e.g. 10 min: experiment time from minute 0 to minute 10). Compare Figs. 3 and 4. (Further details are given in the text)

Experiment	Day	Time interval (min)							
		10	20	30	60	90	120	150	180
1	1	n.s.	n.s.	n.s.	*	**	**	**	**
	2	**	**	**	**	**	**	**	*
	3	**	**	**	**	**	**	**	**
2	1	*	*	**	n.s.	n.s.	n.s.	n.s.	n.s.
	2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	3	*	*	*	*	n.s.	n.s.	n.s.	n.s.
		3	6	9	15	30	60	120	
3	1	n.s.	*	**	**	**	**	n.s.	
	2	**	**	**	**	**	*	n.s.	
	3	**	**	**	**	**	**	*	
4	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	2	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	
	3	**	**	*	n.s.	n.s.	n.s.	n.s.	

*$P \leq 0.05$, **$P \leq 0.01$, n.s. not significant

uation remained very similar to that on day 3. The uptake of *X. luteola* larvae was significantly lower than of the control pupae during the 5-day test period (Table 3, factor "food"). Considering distinct time intervals, significantly more controls were taken up than *X. luteola* larvae during nearly all intervals that were analysed (Table 4).

In summary, the results of experiments 1 and 3 show the following:

1. The tits significantly avoided uptake of *G. tanaceti* and *X. luteola* larvae during the 5-day test period (Table 3, factor "food").
2. Uptake of food (both test and control insects) changed within the 5-day test period. Uptake of food was slow during the 1st day, but accelerated from the 2nd day on (Table 3, factor "day").
3. The degree of avoidance of the chrysomelid larvae significantly increased during the 5-day test period, when considering the time intervals 0–90 min in experiment 1, and 0–30 min in experiment 3 (Table 3, factor "day×food"). As mentioned above, these time intervals were chosen for the ANOVAs because after these intervals about three-quarters of the control insects were eaten, so that there was no longer a good dual choice situation.

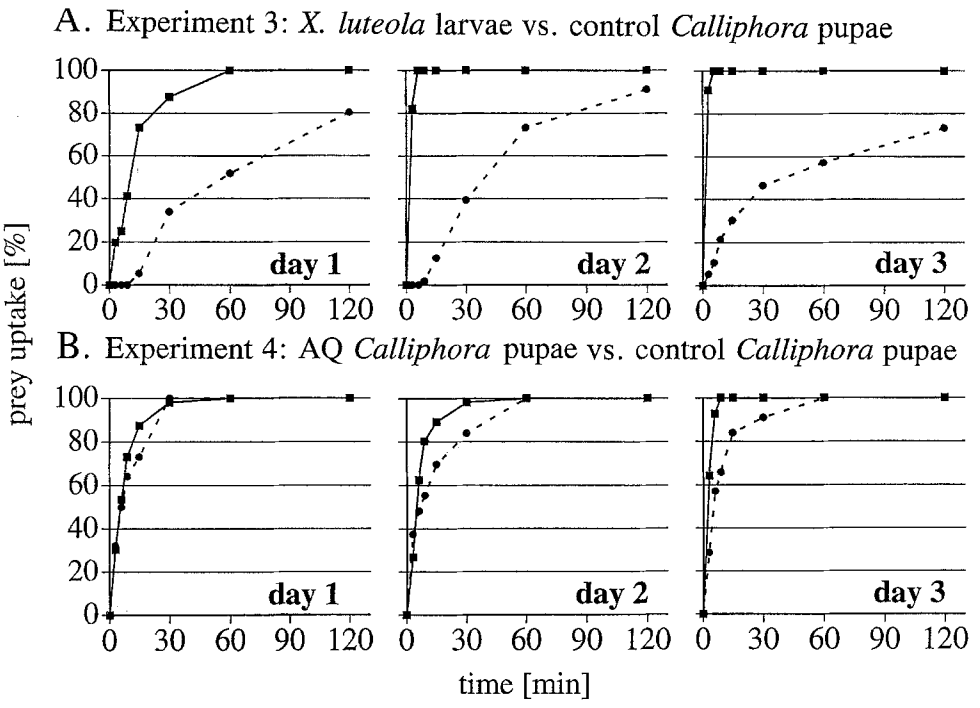

Fig. 4 Uptake of prey offered in experiments **A** 3 and **B** 4 during the 2-h test periods of days 1–3. The figures show the percentages of control pupae (*solid lines*), *Xanthogaleruca luteola* larvae and anthraquinone (*AQ*) treated pupae (both *dashed lines*) taken up by the 8 tits. Prey uptake by the 8 tits was summarized. Further details of experiments 3 and 4 are given in the text

The results of experiments 2 and 4 show the birds' responses towards mealworms and *Calliphora* pupae, treated with anthraquinones. Pecking of AQ-mealworms and AQ-pupae was always followed by swallowing of this prey. This feeding behaviour is in contrast to the feeding behaviour towards *G. tanaceti* and *X. luteola* larvae, which were often pecked and then dropped or partially eaten and then dropped.

On the first day of experiment 2, the birds ate the AQ-mealworms earlier than the controls: after 10, 20, 30, and 60 min significantly more AQ-mealworms were consumed than controls (Fig. 3B, Table 4). After 3 h, nearly all AQ-mealworms (93.8%) were eaten. The control mealworms were offered in petri dishes marked by a red stripe in the middle; the birds were not familiar with these dishes (see Materials and methods). The birds' behavior started to switch on day 2 of this experiment. When comparing the rate of uptake of test and control mealworms during this day at distinct time intervals, no significant differences were observed (Table 4). However, on the 3rd day the tits ate the control mealworms earlier than the AQ-mealworms: during the time intervals 0–10, 0–20, 0–30 and 0–60 min significantly more controls were consumed than treated prey; after e.g. 60 min, less than half the AQ-mealworms had been consumed, but more than 80% of the control food (Table 4). On days 4 and 5 the birds' feeding behaviour towards control and AQ-mealworms showed no more change compared to day 3. Since differences between uptake of test and control insects significantly changed within the 5-day test period, (Table 3, factor "day×food"), the data clearly show that the tits learnt to delay the uptake of AQ-mealworms. Experiment 2 was the only one during which significant differences in feeding behaviour between individual birds were observed (Table 3, factor "bird"). These differences did not occur between the two test species *P. ater* and *P. major*, but between a single *P. ater* individual and the seven other birds. In contrast to the others, this tit did not switch feeding behavior from day 2 to 3, but took up a higher percentage of AQ-mealworms than controls during the first 30 min of each experimental day.

In contrast to experiment 2, no preference for the AQ-prey was observed during the 1st day of experiment 4 (Fig. 4B). As in experiment 2, in experiment 4 the control insects were also offered in petri dishes marked by a red stripe in the middle. However, in experiment 4 the tits already knew these dishes from experiment 2. Both test and control insects were rapidly eaten during the first 30 min of the 1st test day. From the 2nd day on the uptake of AQ-pupae slowed down, so that uptake of AQ- and control pupae differed significantly from each other (Table 3, factor "food").

In summary, the results of experiments 2 and 4 show the following:

1. When AQ-mealworms were simultaneously offered in petri dishes that the tits were familiar with and control mealworms in petri dishes that were not known to the birds, the AQ-prey was eaten faster than the controls during the 1st experimental day. However, within 3 days the tits learnt to consume control prey faster than AQ-prey.
2. When AQ-pupae and control pupae were simultaneously offered in petri dishes that the tits were familiar with, the birds did not distinguish between these food items during the 1st experimental day. However, from the 2nd experimental day on the uptake of AQ-prey was significantly delayed compared to the uptake of controls.

3. Uptake of anthraquinone treated insects was delayed whether they were treated by topical application (experiment 2) or injection (experiment 4).

Discussion

The results clearly show that the tits avoided uptake of *G. tanaceti* and *X. luteola* larvae; these chrysomelid larvae were eaten at a significantly lower percentage than the mealworms and *Calliphora* pupae, respectively. On the other hand, food treated with anthraquinones was eaten at nearly the same percentage as control prey, but the anthraquinone treated food was not eaten as rapidly as the control.

Several factors may account for the birds' reluctance to eat the chrysomelid larvae. First, both larvae of *G. tanaceti* and *X. luteola* display many characteristics the birds were not familiar with: the black colour and hairiness of *G. tanaceti* larvae, and the dark-yellowish colour of the *X. luteola* larvae. From the food the birds were fed with prior to the experiments, they knew the following prey features: a smooth cuticle and light colour from the drone brood, and brown colour from the food mixture. The mealworms and *Calliphora* pupae offered as controls during the experiments also have a smooth cuticula, and light and brown colours, respectively. Thus, the tits were familiar with these food items. Aversion behavior against novelty is widespread in animal species (Thorpe 1963). Experiments with the blue jay, *Cyanocitta cristata*, revealed that this species prefers familiar to novel food (Coppinger 1969). As already mentioned, we also observed prior to the experiments that the tits were reluctant to take up novel food or food that was offered in new dishes.

Second, the hairiness of *G. tanaceti* larvae and the yellowish colour of the *X. luteola* larvae may be features that elicit an innate aversive reaction in tits. Several studies demonstrate the avoidance of hairy food by birds. Field studies of Kristín (1989), for example, revealed that tits (*P. major* and *P. caeruleus*) consumed significantly more unhairy than hairy lepidopteran larvae. Investigations of Tinbergen (1960) on the food composition of wild blue tits showed a very low percentage of hairy lepidopteran larvae eaten compared to nonhairy ones. Not only tits, but also other bird species like paruline warblers, prefer nonhairy to hairy food (Whelan et al. 1989). An innate reluctance to peck at black-and-yellow prey has been suggested by Schuler and Hesse (1985), who studied the uptake of black-and-yellow painted mealworms by naive chicks.

Third, the results obtained by the experiments with food treated with synthetic anthraquinones clearly show that these compounds contribute to the birds' reluctance to eat *G. tanaceti* and *X. luteola* larvae. A detailed comparison of the uptake of AQ- and control food over time revealed significant differences in the birds' feeding behavior towards these two types of food.

In experiment 2, the AQ-prey was consumed more readily than the control during the 3-h test period of the 1st day. However, from the 2nd day on, the birds' feeding behavior towards AQ-mealworms and controls switched; from this day on, the AQ-food was not taken up as readily as the controls. Thus, learning of delayed uptake of AQ-food was observed.

Schuler (1980) studied the impact of the following factors on learning of aversion behavior of starlings:

1. Relearning: an insect, known as palatable prey prior to the experiment, was made distasteful by injection of quinine dihydrochloride and offered to the starlings.
2. New alternative prey: both untreated food and food treated with quinine dihydrochloride were unknown to the birds prior to the experiment.
3. Similarity of alternative prey to palatable food: untreated mealworms and mealworms treated with quinine dihydrochloride were offered.

Schuler's results revealed that all these factors per se significantly retarded learning of aversion to the treated prey. All three factors were also present in our experiment 2, but nevertheless learning to delay the uptake of AQ-food was unambigously shown in this experiment (Table 3, factor "day×food"). Mealworms known as palatable prey to the tits were treated with anthraquinones (relearning). The alternative (control) prey was offered in petri dishes that were marked by a thick red stripe in the middle and therefore were unfamiliar to the birds (new alternative prey). The anthraquinone-treated and untreated food looked identical (similarity of alternative prey to palatable food). Therefore, our experimental design might have retarded learning of delayed uptake of AQ-prey. Since the feeding behavior of the tits towards AQ-food and controls did not change during the 3rd–5th day of experiment 2, we do not expect a stronger aversion behavior towards anthraquinone treated food after a prolonged experiment period.

In experiment 4, the birds' uptake of the AQ-pupae was delayed from the 1st day on (Table 3, factor "food"). In contrast to experiment 2, the birds already knew the marked petri dishes, in which the control food was offered. Thus, the tits readily took up the palatable control pupae in spite of the red marks. Since the birds were exposed to a familiar situation, they did not need to learn delayed uptake of AQ-prey, but were able to show this behaviour right from the beginning (Table 3, factor "day×food", not significant).

Thus, the anthraquinones of galerucine larvae cause a delayed uptake in comparison to insects free of anthraquinones. Such delayed predation, in comparison to alternative food which does not invoke any delay, may be considered as an adaptation to reduce predation pressure. In the field, experienced birds foraging in habitats where both palatable and distasteful prey is present will most probably attack the palatable food first. If no palatable prey is available, the bird might either leave this habitat or start to take up the distasteful prey. In either case there is a large advantage for the unpalatable prey. In the first case, it will not be attacked at all, in the second case it might have sufficient time to escape. For ex-

ample, a prey animal may drop from the plant as soon as it perceives the vibrations caused by birds. When discussing foraging strategies of birds, Schuler (1990) mentioned such prey behaviour as one feature among several others reducing the profitability of a prey for birds. While collecting galerucine larvae in the field, we often observed that they dropped from their hostplants because of disturbance by the collectors. If avian predators hesitate to take up anthraquinone-containing larvae, these larvae gain time to avoid predatory attacks by dropping to the ground.

Up to now, several chrysomelid species have been shown to be distasteful towards birds. Hough-Goldstein et al. (1993) demonstrated that young domestic chickens learnt to avoid both larval and adult Colorado potato beetles (*Leptinotarsa decemlineata*). Larvae of *L. decemlineata* contain the hemolymph protein leptinotarsin, which is toxic against flies and mice when injected, but not when applied orally (Hsiao and Fraenkel 1969). From adult Colorado potato beetles a dipeptide discharged by exocrine glands and toxic against ants, has been identified (Daloze et al. 1986). However, it is unknown whether these compounds cause the obvious distastefulness to chickens.

Begossi and Benson (1988) showed that adults of several alticine species of the genera *Homophoeta*, *Alagoasa*, and *Asphaera* are rejected by young chicks. Evidence for their chemical unpalatability was provided by testing *T. molitor* larvae that were immersed in differently polar fractions of extracts of whole *H. octoguttata* beetles. Of 15 mealworms treated with the low polarity fractions 9 were rejected by the chicks, whereas all mealworms immersed in the polar fractions were consumed.

Rowell-Rahier et al. (1994) demonstrated the defensive activity of the exocrine glandular secretions of *Oreina* species against red-winged blackbirds (*Agelaius phoeniceus*). Some *Oreina* species contain pyrrolizidine alkaloid N-oxides (PAs) in their pronotal and elytral glands, whereas others produce cardenolides. Beetles from which the secretions had been experimentally removed from the glandular openings by repeated stimulation were eaten at a higher percentage than beetles containing secretion in their glands. The birds consumed 21% of the *Oreina* individuals with PAs in their secretion, but 55% of the Oreina specimens with cardenolides in their secretions.

Pasteels et al. (1983) suggested that volatile irritants of insect larvae could be more directed at arthropod predators, whereas nonvolatiles might be mainly directed at birds. The nonvolatile anthraquinones and anthrones of galerucine larvae have been shown now to influence feeding behavior of both ants (Howard et al. 1982; Hilker 1992) and birds.

Acknowledgements We are very grateful to Udo Bauer, Bayreuth, for taking care of the birds. Furthermore, we thank Nathan Rank, Zürich, for helpful criticism of an earlier draft of this manuscript.

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