

# Effects of food plant and group size on predator defence: differences between two co-occurring aposematic Lygaeinae bugs

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**Abstract.** 1. The role of food plant and aggregation on the defensive properties of two aposematic sympatrically occurring seed bugs, *Tropidothorax leucopterus* and *Lygaeus equestris* (Lygaeinae, Heteroptera), was investigated. Larvae reared on seeds either of their natural host plant *Vincetoxicum hirundinaria* (Asclepiadaceae) or of sunflower *Helianthus annuus* were subjected to predation by chicks.

2. The two species differ in their dependency on the host for their defence. *Lygaeus equestris* was better defended on its natural host plant than on the alternative food, as indicated by fewer attacks, lower mortality, and predator avoidance after experience. No such effect of food plant could be found for *T. leucopterus*, suggesting the existence of alternative defences in this species.

3. The number of attacks was lower when host plant-fed larvae of both species were presented in groups.

4. The discussion concerns how major components of an aposematic syndrome, such as host plant chemistry, insect colouration, and aggregation, are integrated with other life-history traits to form alternative lifestyles in *L. equestris* and *T. leucopterus*.

**Key words.** Aggregation, Asclepiadaceae, defence, host plant, life history, Lygaeinae, *Lygaeus equestris*, palatability, *Tropidothorax leucopterus*.

## Introduction

Many species of milkweed bugs (Heteroptera: Lygaeidae: Lygaeinae) are aposematically coloured and feed on various toxic plant taxa within the families Asclepiadaceae, Apocynaceae, and Senecionaceae (Slater & Sperry, 1973; Solbreck *et al.*, 1990). It is generally believed that these insects are dependent on their host plants for the sequestration of substances used in their own defences (Scudder & Duffey, 1972; Aldrich, 1988), and some studies have been able to relate the content, for example, cardiac glycosides (cardenolides) in milkweed bugs to that in their asclepiadaceous food plants (Duffey & Scudder, 1972; Isman *et al.*, 1977; Duffey *et al.*, 1978; Vaughan, 1979). For example, *Oncopeltus fasciatus*, when reared on cardenolide-free sunflower seeds, did not contain any cardenolides (Scudder & Duffey, 1972).

There is, however, considerable variation in the degree to which milkweed bugs utilise cardenolide-containing plants and some species tend to be polyphagous, feeding also on plants lacking these and other poisonous substances. It would then be expected that there is a potential cost associated with using alternative nonpoisonous plants. For instance, McLain and Shure (1985) demonstrated that *Neacoryphus bicrucis* became palatable to anole lizards when fed seeds lacking the pyrrolizidine alkaloids of its natural host plant.

Gregariousness is a common feature among aposematic insects in general and in milkweed bugs in particular. There is, however, considerable variation also in this trait, from species that live solitarily throughout their life, to species that aggregate only during a particular phase in the life cycle, to species that aggregate during all life stages. Gregariousness and defence properties may be linked in several ways (Sillén-Tullberg & Leimar, 1988); for instance, aggregation may give a cumulative effect of noxious substances (Aldrich & Blum, 1978) or an increased effect of the aposematic signal (Gamberale & Tullberg, 1996a, 1998). If gregariousness has evolved as a

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response to predation, it would be expected that the relative benefit of this trait *vis-à-vis* a predator would be greater in gregarious species than in solitary species.

In South Central Europe, two species of lygaeid bugs, *Tropidothorax leucopterus* Goeze and *Lygaeus equestris* L., co-occur on their asclepiadaceous host plant *Vincetoxicum hirundinaria* Medicus, a species that, although lacking cardenolides (Dobler *et al.*, 1998), is known to contain various other presumably toxic substances (Stöckel *et al.*, 1969a,b,c; Eggerman & Bongers, 1972; Eibler *et al.*, 1995). *Tropidothorax leucopterus* is monophagous on *Vincetoxicum* whereas *L. equestris*, although preferring this plant, may feed on other plants, some of which evidently lack toxic substances (Solbreck & Kugelberg, 1972; Kugelberg, 1973a,b, 1974; Anderson & Solbreck, 1991). The two species also differ with regard to the plant parts being used. Thus, *T. leucopterus* uses ovulae and seeds but can also develop on vegetative parts, whereas *L. equestris* depends on ovulae or seeds to complete its larval development. The two species also differ in their tendency to aggregate with conspecifics. *Tropidothorax leucopterus* is highly gregarious during all life stages whereas *L. equestris*, although sometimes forming large overwintering aggregations as an adult, is considerably less gregarious than *T. leucopterus* as a larva. Thus, on the host plant, *T. leucopterus* often forms very large clusters whereas *L. equestris* occurs solitarily or in tiny groups (Anderson & Solbreck, 1991).

The study reported here deals with the question of whether the differences in host plant utilisation and gregariousness shown by *T. leucopterus* and *L. equestris* can be related to their antipredatory defences. The benefits conferred on larvae by feeding on the toxic primary host plant vs. a nontoxic alternative and the advantage of forming aggregations were investigated using naive chicks as predators.

## Materials and methods

### Predators and prey

Domestic chicks *Gallus gallus domesticus* were used as predators. The chicks arrived from the hatchery in batches of about 40 individuals. On arrival, they were less than 20 h old and had not eaten. Each batch was housed in a 100 × 55 × 20 cm cage with wooden sides, steel-net floor, and a roof made of wood and chicken wire. The cage was heated with a 60 W carbon light bulb and the floor of the cage was covered with sawdust. All chicks were fed chick starter crumbs and water, and from their second day on they were also fed live mealworms *Tenebrio molitor*. Throughout the experiments, birds in each batch were divided evenly among treatment groups.

Live fourth-instar larvae of the seed bugs *T. leucopterus* and *L. equestris* were used as prey. Both species were cultured from specimens collected in northern Italy. Larvae of each species were reared on seeds from either the host plant *Vincetoxicum hirundinaria* or the nontoxic *Helianthus annuus*. Each insect species was reared at its optimum temperature with regard to egg and larval survival: *T. leucopterus* at 27 °C and

*L. equestris* at 32 °C (Anderson & Solbreck, 1991). The light:dark regime was 17:7 h for both species. In *T. leucopterus*, all larvae, independent of size, instar, and food plant are of the same colouration (at least to the human eye), being bright red with black head, legs, and wing buds. The colouration of *L. equestris* larvae is more variable and depends to a certain degree on their food. Thus, larvae reared on seeds of the host plant are more reddish whereas those reared on sunflower seeds tend towards orange or greyish red; head, legs, and wing buds are black as in *T. leucopterus*. Both species have been used in previous studies of aposematism (Sillén-Tullberg *et al.*, 1982; Gamberale & Tullberg, 1996b), in which it was shown that they are distasteful to birds.

Fourth-instar larvae of both species were larger (mean ± SD) when fed on *H. annuus* than when fed on host plant seeds (*T. leucopterus* on *H. annuus*: 0.51 ± 0.04 cm, *n* = 9; on *V. hirundinaria*: 0.48 ± 0.03 cm, *n* = 10; *t* = 2.67, d.f. = 17, *P* < 0.05; *L. equestris* on *H. annuus*: 0.68 ± 0.03 cm, *n* = 10; on *V. hirundinaria*: 0.60 ± 0.04 cm, *n* = 9; *t* = 3.97, d.f. = 17, *P* < 0.001). Note also that *L. equestris* is the larger species.

### Experimental procedure

The experiments took place in an open arena similar to the cage in which the chicks were housed. The floor of the arena was covered with a transparent plastic sheet and the background colouration was that of sawdust placed under the steel-net floor. The experimental room was lit by daylight lamps in order to cover the natural light spectrum. The chicks were tested in pairs because they easily become distressed when alone. The testing started on the birds' fifth day.

Insects were presented in eight 5-cm diameter Petri dishes placed 20 cm apart in two rows in the testing arena. A thin layer of Fluon<sup>®</sup> (Whitford Plastics Ltd, Runcorn, U.K.) was applied to the rim of each dish to prevent insect escape. In order for the birds to habituate to the testing arena and to the testing procedure, they were served single mealworms in the eight Petri dishes in a first trial. This trial could be carried out with single birds; their interest in this particular kind of food overruled the negative experience of being alone. The learning procedure was fast and the birds generally devoured all the mealworms in less than 1 min.

The chicks were divided into six experimental groups with regard to test insects, each group consisting of 11 or 12 pairs of chicks. Each pair was presented with the same kind of prey in each of the eight Petri dishes. The tests were carried out on two consecutive days. Each daily trial lasted 6 min, which gave the chicks time to explore the arena. The experimental groups were: single *T. leucopterus* reared on *V. hirundinaria* (*n* = 12), single *T. leucopterus* reared on *H. annuus* (*n* = 12), single *L. equestris* reared on *V. hirundinaria* (*n* = 12), single *L. equestris* reared on *H. annuus* (*n* = 11), groups of *T. leucopterus* reared on *V. hirundinaria* (*n* = 12), and groups of *L. equestris* reared on *V. hirundinaria* (*n* = 12). In the group treatment, each of the eight Petri dishes contained nine insects. On the first test day, the birds were inexperienced to the experimental prey.

The behaviour of individual birds in the pair and their interaction with prey in the separate Petri dishes were recorded during each trial. For each bird, records were taken on the number of attacks and whether attacked insects were killed or eaten. Insects with ruptured cuticles were considered mortally wounded. The plastic sheet in the arena was cleaned after each pair of chicks was tested.

Because the two birds in a pair may interact during a trial, the pooled behaviour of the two birds was regarded as an independent observation.

In order to test whether there was an effect of experience, the number of attacks on the two test days was compared using data from only one individual in each pair, i.e. the first bird to attack on day 1.

## Results

### Effect of food plant

Food plant species affected the defensive properties of the two insect species in different ways. In *L. equestris*, there was a cost of feeding on the nontoxic plant (*H. annuus*), as evidenced by higher attack rate and mortality as well as by higher feeding rate by the chicks than when reared on the toxic plant (*V. hirundinaria*). There was no such cost in *T. leucopterus* (Fig. 1).

An ANOVA showed a significant interaction between insect species and food on the number of attacks (Table 1). The number of attacks was significantly higher on *L. equestris* when reared on *H. annuus* than when raised on *V. hirundinaria* seeds (Tukey test:  $P < 0.05$ ), whereas there was no significant food plant effect in *T. leucopterus* (Tukey test:  $P = \text{NS}$ ) (Fig. 1a).

The role of experience was also affected by treatment. There was evidence for individual chicks avoiding *V. hirundinaria*-reared *L. equestris*; they attacked significantly fewer bugs (mean  $\pm$  SD) on the second test day ( $1.82 \pm 1.54$ ,  $n = 11$ ) than on the first test day ( $2.81 \pm 1.25$ ) (paired  $t$ -test:  $t = 2.47$ , d.f. = 10,  $P < 0.05$ ). No such effect was found for *L. equestris* reared on *H. annuus* seeds ( $t = 1.04$ , d.f. = 10,  $P = \text{NS}$ ). For *T. leucopterus*, there was no evidence of avoidance in either treatment; there was no significant decrease in attacks from the first to the second test day (*V. hirundinaria*:  $t = 0.42$ , d.f. = 11,  $P = \text{NS}$ ; *H. annuus*:  $t = 0.15$ , d.f. = 10,  $P = \text{NS}$ ).

*Lygaeus equestris* suffered higher mortality when reared on *H. annuus* seeds than on *V. hirundinaria* seeds ( $t = 2.85$ , d.f. = 21,  $P = 0.01$ ), whereas mortality in *T. leucopterus* was lowest on *H. annuus*-fed bugs ( $t = 2.31$ , d.f. = 22,  $P < 0.05$ ) (Fig. 1b).

Only in one of the four treatments, i.e. *H. annuus*-fed *L. equestris*, was a substantial number of prey items eaten (Fig. 1c). Only two individual *T. leucopterus* were eaten during the whole experiment.

The enhanced mortality of *H. annuus*-fed *L. equestris* (Fig. 1b) seems entirely due to increased consumption (Fig. 1c), however it should be noted that even though some chicks found these bugs reasonably edible, consumption rate on

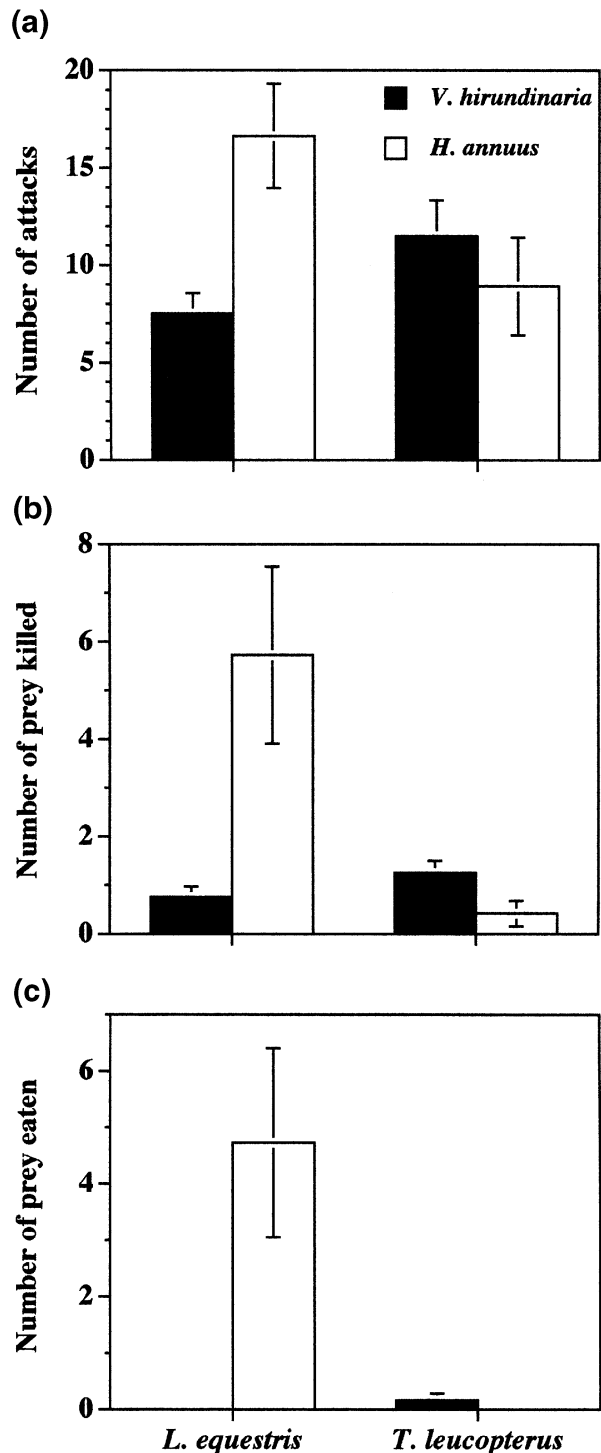


Fig. 1. Mean ( $\pm$  SE) number of fourth-instar larvae of *Lygaeus equestris* and *Tropidothorax leucopterus* (a) attacked, (b) killed, and (c) consumed by chicks ( $n = 11$  or  $12$  for each treatment). Insects had been reared on either *Vincetoxicum hirundinaria* or *Helianthus annuus* (sunflower) seeds.

**Table 1.** Repeated measures ANOVA for number of attacks on fourth-instar larvae of *Tropidothorax leucopterus* or *Lygaeus equestris* fed on seeds of *Vincetoxicum hirundinaria* or *Helianthus annuus*.

Source of variation	d.f.	MS	F	P
Species	1	20.29	0.79	0.380
Food	1	62.98	2.44	0.125
Day	1	5.73	0.57	0.456
Species × food	1	201.45	7.82	0.008
Residual interactions	3	9.69	0.96	>0.25
Error	43	25.76		

these insects was still much lower than on fully palatable prey such as mealworms.

#### Effect of group size

There were fewer attacks on *V. hirundinaria*-reared fourth-instar larvae when they were presented in groups of nine than when they were presented as single individuals in each Petri dish (Table 2). The number of attacks also differed between the species, *T. leucopterus* being attacked more than *L. equestris* (Table 2; Fig. 2).

There was no effect of experience in individual chicks when they were presented groups of either prey species; there was no significant decrease in the number of attacks from the first to the second test day (*L. equestris*:  $t=0.0$ , d.f. = 6,  $P=NS$ ; *T. leucopterus*:  $t=0.37$ , d.f. = 9,  $P=NS$ ; paired-sample  $t$ -tests).

## Discussion

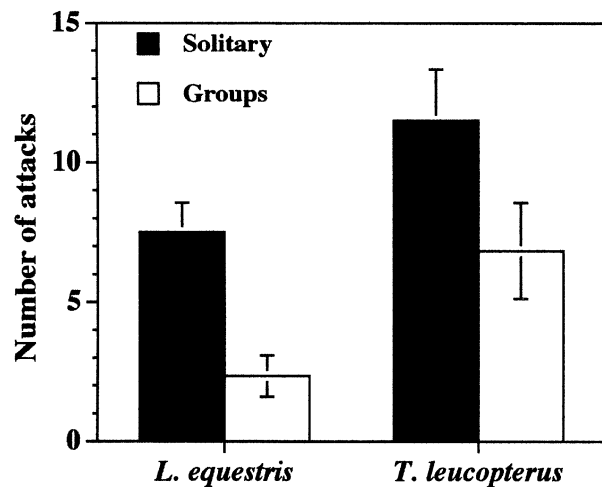
### Host plant

Species that sequester toxins or noxious substances from their host plants generally show a high degree of plant dependence with regard to their antipredatory defences (Brower, 1969; McLain, 1984; Peterson *et al.*, 1987; Ritland, 1994). Both species in the study reported here were relatively well defended (compared with the fully palatable mealworms) when reared on the nontoxic food, sunflower seeds. This suggests the occurrence of two lines of defence – one dependent on host plant, the other independent of host plant. It is possible that plant-independent defences stem from the volatile substances and contact poisons (Remold, 1963) generally occurring in heteropteran stink glands. The fact that larvae of both *L. equestris* and *T. leucopterus* emit a strong smell when disturbed suggests that their stink glands are effective. Such defences are likely to have effects against birds, although the effectiveness may be low against some bird predators (Alcock, 1973; Schlee, 1986).

The association between defence and food intake differs between the two species. Defence in *L. equestris* is food dependent and *V. hirundinaria*-fed *L. equestris* were attacked

**Table 2.** Repeated measures ANOVA for number of attacks on fourth-instar larvae of *Tropidothorax leucopterus* or *Lygaeus equestris* fed on seeds of *Vincetoxicum hirundinaria* and presented in group sizes of one or nine individuals.

Source of variation	d.f.	MS	F	P
Species	1	108.38	8.98	0.004
Group size	1	145.04	12.01	0.001
Day	1	0.0	0.0	1.0
Species × group size	1	0.37	0.03	0.861
Residual interactions	3	3.78	1.16	>0.25
Error	44	12.07		



**Fig. 2.** Mean ( $\pm$  SE) number of attacks by chicks on fourth-instar larvae of *Lygaeus equestris* or *Tropidothorax leucopterus*, presented singly or in groups of nine ( $n=11$  or  $12$  for each treatment). All insects were reared on *Vincetoxicum hirundinaria*.

less and had a considerably lower mortality than their sunflower-reared conspecifics. One reason for the low attack rate may be that larval colouration is affected by food; *V. hirundinaria*-reared larvae are bright red whereas sunflower-fed larvae are paler (see above). The differences in both attack and mortality rates are, however, affected primarily by a difference in palatability, as indicated by the fact that many birds actually devoured the sunflower-reared *L. equestris*. Because this species utilises other plants when *V. hirundinaria* seeds are in short supply, its palatability is likely to vary in the field. Thus, polyphagy seems to involve a cost in terms of weaker defence in this species.

There was no indication in this experiment that the natural host plant enhances antipredator defences in *T. leucopterus*, because there was no significant difference in the number of attacks on larvae reared on the two types of food. The fact that *T. leucopterus* is quite unpalatable in spite of being reared on sunflower seeds suggests that the bug is capable of producing its own defence substances. Surprisingly, *T. leucopterus* survived attacks better when reared on sunflower. This is probably an effect of their somewhat larger size (see above),

which might have rendered them more resistant to injury. In a previous experiment, it was shown that size causes lower attack rate as well as lower mortality in an attack (Gamberale & Tullberg, 1996b). Similarly, the lower attack rate on *L. equestris* than on *T. leucopterus* when both were raised on *V. hirundinaria* (Table 2, Fig. 2) may be an effect of the larger size of the former (see above).

The differences shown between *L. equestris* and *T. leucopterus* suggest that the defences in lygaeid bugs are more complex than previously thought (see also Aldrich *et al.*, 1997), involving variations in plant use and concomitant size and colour effects as well as host-independent antipredatory defences. Synthesis of toxic compounds has been shown to occur in some chrysomelid genera (Pasteels & Daloze, 1977; Dobler *et al.*, 1998) but it is not known whether this occurs in the Lygaeinae.

### Aggregation

Predators were less inclined to attack aggregated bugs than solitary bugs (Table 2, Fig. 2). Aggregation in insects may have various functions, including increased ability to escape attacks (Vulinec, 1990), but the insects in the present experiment had no chance to flee. In previous experiments, naive chicks readily attacked groups of nonaposematic (brown) lygaeid bugs but were reluctant to attack groups of aposematic lygaeid bugs. Thus, it was concluded that gregariousness may enhance the deterrent effect of an aposematic colouration (Gamberale & Tullberg, 1998). The increased signal effect may be a contributing factor in explaining the high incidence of aggregation in aposematic heteropterans (e.g. Aldrich, 1988).

### Aposematism and lifestyles

*Lygaeus equestris* and *T. leucopterus* have fundamentally different ways of exploiting *V. hirundinaria* as a host plant. *Tropidothorax leucopterus* is essentially monophagous on this host but it has a wide spectrum with regard to the plant parts being used. Furthermore it has larger egg clutches, is less inclined to fly away from a host plant patch, and is more starvation tolerant. This leads to higher local densities of *T. leucopterus* than of *L. equestris*, which, in combination with a higher tendency to aggregate and to forage up on the plant, provides a more drastic exposure of its aposematic colouration. *Lygaeus equestris*, on the other hand, is more specialised on seeds but has a wider spectrum with regard to host plant and habitat. It is more migratory and utilises seeds on both plant and ground (Kugelberg, 1977; Anderson & Solbreck, 1991). Thus, *L. equestris* is presumably exposed to a wider spectrum of host plant chemicals and to a wider range of habitats, which in effect means more variation with regard to background colours and, at least in the adult stage, to increased exposure to inexperienced predators. In this connection, it is worth noting that *L. equestris* is also more variable in colouration. This variation has both genetic (Sillén-Tullberg, 1985; Solbreck *et al.*, 1989) and environmental (this study) components.

It was expected that *T. leucopterus*, being monophagous, would have the least need to synthesise its own protective substances. This expectation, however, rests on the assumption that the supply of toxic substances in the plant does not vary too much in time and space. In other species of Asclepiadaceae (Nelson *et al.*, 1981; Malcolm *et al.*, 1989) there may be variation among plant individuals giving rise to a palatability spectrum (Pough *et al.*, 1973), as shown by Björkman and Larsson (1991). This is, however, likely to be of minor importance for the antipredatory defence in *T. leucopterus*.

Host plant chemistry, insect colouration, and aggregation are major components of an aposematic syndrome. *Lygaeus equestris* and *T. leucopterus* illustrate how these components are integrated with other life-history traits to form alternative lifestyles. In both species, gregariousness reduces the number of attacks in predator encounters, but only *T. leucopterus* form large larval aggregations in the field. The reason might be that a combination of aposematic colouration and an exposed lifestyle up on the plant makes it less costly, in terms of detectability, to be aggregated (Sillén-Tullberg & Leimar, 1988). Thus, because each individual is quite conspicuous in itself, the increased detectability in a group might be small and outweighed by the decreased attack rate at discovery. Moreover, food utilisation *per se* may have played an important role in the gregarious habits of this species, in that it allows for a sedentary lifestyle. This should be seen in contrast to the lifestyle of *L. equestris* larvae, which involves a continuous search for dispersed seeds on the ground. Always having a safe defence like *T. leucopterus* may facilitate the maintenance of bright colouration and massive gregariousness, whereas the wider host plant spectrum and vagrant lifestyle of *L. equestris* puts less premium on these traits.

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