Evidence for a more effective signal in aggregated aposematic prey

GABRIELLA GAMBERALE & BIRGITTA S. TULLBERG

Department of Zoology, University of Stockholm, Sweden

Abstract. Aposematic prey tend to live gregariously. A recent study using artificial prey has shown that a gregarious life style can be advantageous by generating faster avoidance learning in predators (Gagliardo & Guilford 1993, *Proc. R. Soc. Lond. Ser. B*, **286**, 149–150). However, a predator may react differently to artificial and live prey. This study investigates whether chicks, *Gallus gallus domesticus*, react differently towards aggregations and solitary individuals of the seed bug *Spilostethus pandurus* (Heteroptera: Lygaeidae). There was no significant difference in the speed of avoidance learning between chicks presented with grouped prey and chicks presented with solitary prey. The aggregated prey did, however, generate greater unconditioned aversion than prey presented singly. This indicates that a possible advantage of grouping in aposematic prey is a more effective aposematic signal. The greater unconditioned aversion is comparable to the generally greater initial reluctance of predators to attack aposematic than cryptic prey.
© 1996 The Association for the Study of Animal Behaviour

Gregariousness is more common in aposematic than in palatable and cryptic species (Fisher 1958; Turner 1975; Järvi et al. 1981a). Phylogenetic analyses also show that the presence of aposematism makes gregariousness more likely to evolve in lepidopteran larvae (Sillén-Tullberg 1988, 1993; Tullberg & Hunter 1996).

There are several ways that gregariousness could be beneficial to aposematic prey by reducing predation. When predators learn by experience not to attack unprofitable prey, possible prey would benefit by gathering together in as few predator home ranges as possible and hence reducing the risk of encountering inexperienced predators (Leimar et al. 1986). Moreover, for prey that are small in relation to their potential predators, unprofitability can act as a predator satiation mechanism in the sense that it limits the number of prey taken. Such limitation on the number of prey that are attacked creates a dilution effect which makes it advantageous for prey to stay in large groups (Sillén-Tullberg & Leimar 1988). Furthermore, gregariousness could enhance the effectiveness of, for instance, chemical defence (Cott 1940; Tostowaryk 1972;

Treisman 1975; Aldrich & Blum 1978; Howard et al. 1983).

One commonly given reason for prey forming aggregations is that they are more deterrent and make predators less likely to attack (Cott 1940; Edmunds 1974), but this signal effect has not yet been experimentally verified (Sillén-Tullberg 1990, 1992; Cooper 1992). That chicks learn faster, and more strongly, to avoid aggregated than solitary artificial prey was shown by Gagliardo & Guilford (1993). However, their results suggest that the mechanism that enhances the avoidance learning process is not the aggregation per se but that birds are able to see the stimulus as, or immediately after, the noxious taste is perceived. Also, it is possible that the use of artificial prey (chick crumbs, which are usually eaten whole) instead of live prey could have influenced the outcome of this experiment. For example, the suggested mechanism may work as well for solitary live prey able to survive predator attacks (Gagliardo & Guilford 1993), and may not be improved by aggregation.

For the above reasons, we decided to use live aposematic prey to determine whether aggregating makes the aposematic signal more effective. We compared unconditioned aversion and avoidance learning with solitary and aggregated prey.

Correspondence: G. Gamberale, Department of Zoology, University of Stockholm, S-10691 Stockholm, Sweden (email: gabriella-gamberale@zoologi.su.se).

METHODS

Subjects and Housing Conditions

As predators in the experiment we used male domestic chicks, *Gallus gallus domesticus*, of the Shaver 288 breed. The chicks arrived in batches of 20, from a commercial hatchery. On arrival they were less than 10 h old and had not yet eaten. The 20 chicks were housed together in a cage measuring $100 \times 55 \times 20$ cm with wooden sides and a steel-net floor covered with sawdust. The roof of the cage was composed half of wood and half of chicken wire. Heat was provided by a 60 W carbon light bulb. The chicks were fed chick starter crumbs, mealworms and water.

As prey we used the quite conspicuous fifth instar larvae of the bug *Spilostethus pandurus* (Heteroptera: Lygaeidae). They have a bright red body with black wing buds and head. They are likely to be distasteful because they secrete defensive compounds (Remold 1963; Staddon 1979) and the experimental birds showed signs of aversion (forcible head shaking and bill wiping) on several occasions when handling the larvae. In nature the bugs are solitary and are therefore not expected to have any synchronized communal defence. The insects were kept in cultures with an 18:6 h light:dark regime and at a temperature of 30°C. They were reared on a diet of husked sunflower seeds and water.

Experimental Arena and Procedure

Each batch of birds was tested on the second day after arrival, at an approximate age of 45–55 h. The testing was conducted in three arenas made of the same materials as the housing cages. Part of each cage was screened off with cardboard leaving a testing-floor size of 30×55 cm. The chicks were tested in pairs because they become distressed and lose interest in eating when alone in a new environment (cf. Dawkins 1971; Roper & Wistow 1986). Approximately 1 h before testing, the chick-pairs were isolated in the experimental arena where they had free access to water but no food.

We tested 74 chick-pairs. Each batch of chicks was divided in half: one half was presented with solitary insects (total $N=37$) and the other with groups of insects (total $N=37$). The insect groups were arranged so that the chicks were able to attack only one insect. Two petri dishes were placed on top of each other and taped together. The bottom dish contained seven inaccessible larvae and an eighth, accessible larva was placed in the top dish, making visible a group of eight. When solitary insects were presented, one individual was placed in the top dish and the bottom dish was left empty. The rim of each top dish was smeared with a thin layer of Vaseline to prevent the insect escaping. In each trial a mealworm was presented simultaneously with the test insects as a control. The mealworm was placed in the same kind of petri dish arrangement as the test insects.

The testing of a chick consisted of 10 1-min trials. The trials started every second minute, leaving 1 min between them. The first chick of a pair to attack a test insect became the 'experimental bird', making the other one automatically a 'companion bird'. We noted the behaviour of both the experimental bird and the companion bird, but used only data from the experimental birds in the analyses. For a trial to count, the birds had to attack the mealworm and/or the test insect. If the experimental bird attacked the test insect, that trial was terminated as soon as the bird had ceased handling the insect. After each attack, we looked at the prey: if the cuticle was ruptured the insect was considered dead.

Statistical Analysis

We compared the number of attacks on solitary and gregarious prey and tested the difference using a contingency table. The avoidance learning in birds that attacked prey was measured as the number of attacks during the experiment. We also noted the proportion of birds that attacked more than once. We measured the chicks' unconditioned aversion as the total number of birds attacking gregarious and solitary insects. The difference in attack probability between the two groups was tested using a contingency table.

RESULTS

The chicks behaved differently towards the two presentation types. Over the whole experiment, there were about twice as many attacks on solitary prey as on gregarious prey (Fig. 1). In 370 trials with gregarious prey there were 27 attacks and in 370 trials with solitary prey, there were 55 attacks
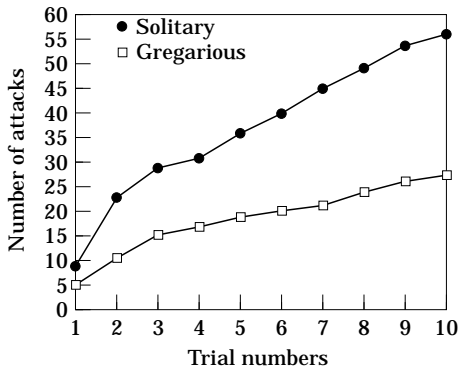
Figure 1. The cumulative number of attacks by chicks on solitary and gregarious *S. pandurus* larvae over 10 trials.
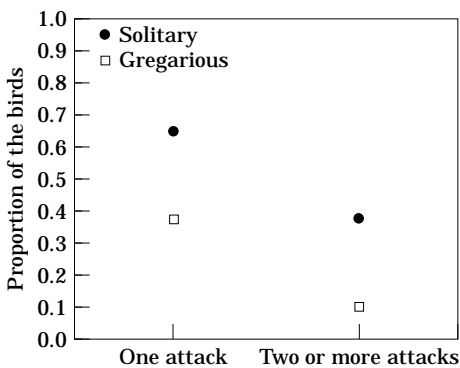
Figure 2. The proportion of chicks that attacked at least once, and that attacked twice or more, when presented with either solitary or gregarious *S. pandurus* larvae.

($\chi^2=10.752$, $df=1$, $P=0.001$). The difference in attack frequency between the two presentation types could be due to (1) faster avoidance learning in predators when the prey were gregarious and (2) greater unconditioned aversion towards groups of aposematic prey.

The majority of the chicks that attacked, independent of presentation type, attacked only once and avoided the prey during the remaining experimental trials (Fig. 2). We could not detect any significant difference in avoidance learning between the two treatments. There was no difference in the number of attacks by the birds during testing: of all birds that did attack, the number of attacks needed to learn was 2.25 ± 0.37 ($\bar{X} \pm$ SE; $N=24$) for the solitary treatment, and for birds receiving the aggregated treatment 1.93 ± 0.39 ($N=14$; $U=191.5$, $P=0.45$). Another way to

determine if there is any difference in avoidance learning between the birds in the two treatments is to compare how many birds attacked a second time within five trials of an attack. Among birds that attacked prey a somewhat greater proportion of birds that were presented with solitary prey attacked a second time (13 out of 24) than birds that were presented with aggregated prey (4 out of 14), although the difference is not significant ($\chi^2=2.343$, $df=1$, $P=0.13$).

The greater attack frequency on the solitary prey could rather be explained by the greater initial deterrent effect that aggregated prey had on the chicks. This was shown as a greater reluctance to attack at all among birds presented with aggregated prey than among birds presented with solitary prey (Fig. 2): 24 birds out of 37 attacked the solitary prey and only 14 out of 37 attacked the aggregated prey ($\chi^2=5.409$, $df=1$, $P=0.02$).

If predators are more reluctant to attack aggregated prey, and if this represents a general reluctance and carefulness when dealing with such prey, a reasonable expectation would be that, given an attack, they would also handle aggregated prey more carefully. Such coupling between reluctance to attack and carefulness when handling prey, as judged from prey mortality, has been found when comparing aposematic and cryptic prey of the same species (Sillén-Tullberg 1985). However, this was not the case in the present experiment. It seems as if chicks that were presented with gregarious prey attacked more forcefully, when they finally attacked, than did birds that were presented with solitary prey. Out of the 27 gregarious prey that were attacked during the whole experiment 15 were ruptured and of the 55 solitary prey that were attacked only 17 were ruptured ($\chi^2=4.623$, $df=1$, $P=0.03$).

DISCUSSION

For a gregarious life style to evolve as a predator defence strategy, the benefits have to exceed the costs, the most obvious of which is the increased risk of discovery. This experiment indicates that a possible advantage of group living in aposematic prey is an increase in the effectiveness of the aposematic signal. The significant effect of gregariousness on predator behaviour was not an increase in avoidance learning, but a greater unconditioned aversion when the aposematic prey

was presented in groups. This effect is comparable to the generally greater initial reluctance of predators to attack aposematic than cryptic prey, the 'novelty effect', that was first demonstrated by Coppinger (1969, 1970). However, in the case of gregariousness, the effect is not due to a difference in coloration but possibly to the amount of coloration visible. If this is so, one would expect the same effect to show in predators exposed to prey with the same colour but of different size, and also a greater avoidance of larger than of smaller aggregations of prey of the same size.

Larvae in the gregarious condition did not survive better than solitary larvae; indeed, a higher proportion were injured. A possible explanation for this somewhat surprising finding is that only the hungrier or more aggressive chicks were not deterred initially by the gregarious treatment. Further experiments are warranted to show whether the phenomenon is of regular occurrence.

The lower attack probability on grouped prey can only be an effect of the aggregation itself regardless of any subsequent effect that aggregation may have on avoidance learning. As Gagliardo & Guilford's (1993) experiment indicated, it was not the appearance of an aggregation that was the important stimulus for the enhanced aversion learning when prey were grouped, but the opportunity for the chick to see the prey's coloration when, or immediately after, noticing the prey's distastefulness. Gagliardo & Guilford (1993) suggested that prey can possess other features besides aggregating, such as resilience to ingestion, large size and expendable parts, that can allow warning coloration to be perceived in connection with the noxious taste. One reason why no clear increase in avoidance learning could be detected among birds that were presented with gregarious prey could be that the mechanism worked as well for solitary prey as for prey presented in groups. Thus, the fact that so many of the distasteful insects survived the predator attacks may have been sufficient for the birds to associate the prey's coloration with unpalatability. However, since there was a tendency for more birds to attack more than once when given solitary larvae, we cannot exclude the possibility of a difference in speed of avoidance learning that could be detected only with a larger sample size.

Our experiment shows that gregariousness can indeed lower the attack frequency on aposematic insects, but the question is under what circumstances this happens. For instance, in experiments with the aposematic larvae of *Papilio machaon*, great tits, *Parus major*, and quails, *Coturnix coturnix*, tended to attack groups more than solitary individuals (Sillén-Tullberg 1990, 1992). This indicates that the effect found in the present study is not necessarily general for aposematic prey. One possible reason for the difference between experiments could be that different predators were used. However, another reason could be differences between the aposematic colorations of the two prey types. For instance, the larvae of *P. machaon* were presented on twigs of their host plant, *Peucedanum palustre*, which renders them quite cryptic at a distance (Järvi et al. 1981b). Thus, in this situation aggregated prey might not increase any signal effect of the coloration but may be discovered more rapidly. In the present experiment, on the other hand, the red and black larvae of *S. pandurus* against the background of sawdust were clearly conspicuous (at least to the human eye), and it is possible that such a situation is necessary to make predators more hesitant towards attacking aggregations than solitary individuals.

ACKNOWLEDGMENTS

REFERENCES

Aldrich, J. R. & Blum, M. S. 1978. Aposematic aggregation of a bug: the defensive display and formation of aggregations. *Biotropica*, **10,** 58–61.

Cooper, W. E. 1992. Does gregariousness reduce attacks on aposematic prey? Limitations of one experimental test. *Anim. Behav.*, **43,** 163–164.

Coppinger, R. P. 1969. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies. I. Reactions of wild-caught adult blue jays to novel insects. *Behaviour*, **35,** 4–60.

Coppinger, R. P. 1970. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies. II. Reactions of naïve birds to novel insects. *Am. Nat.*, **104,** 323–335.

Cott, H. B. 1940. *Adaptive Coloration in Animals.* London: Methuen.

Dawkins, M. S. 1971. Shifts in 'attention' in chicks during feeding. *Anim. Behav.*, **19**, 575–578.

Edmunds, M. 1974. *Defence in Animals: a Survey of Anti-predator Defences*. Harlow: Longman.

Fisher, R. A. 1958. *The Genetical Theory of Natural Selection*. 2nd edn. New York: Dover Publications.

Gagliardo, A. & Guilford, T. 1993. Why do warning-coloured prey live gregariously? *Proc. R. Soc. Lond. Ser. B.*, **286**, 149–150.

Howard, D. F., Blum, M. S. & Fales, H. M. 1983. Defense in thrips: forbidding fruitiness of a lactone. *Science*, **220**, 335–336.

Järvi, T., Sillén-Tullberg, B. & Wiklund, C. 1981a. Individual versus kin selection for aposematic coloration: a reply to Harvey and Paxton. *Oikos*, **37**, 393–395.

Järvi, T., Sillén-Tullberg, B. & Wiklund, C. 1981b. The cost of being aposematic. An experimental study of predation on larvae of *Papilio machaon* by the great tit *Parus major*. *Oikos*, **36**, 267–272.

Leimar, O., Enquist, M. & Sillén-Tullberg, B. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. *Am. Nat.*, **128**, 469–490.

Remold, H. 1963. Scent-glands of land-bugs, their physiology and biological function. *Nature, Lond.*, **198**, 764–768.

Roper, T. J. & Wistow, R. 1986. Aposematic colouration and avoidance learning in chicks. *Q. Jl Exp. Psychol.*, **38(B),** 141–149.

Sillén-Tullberg, B. 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia (Berl.)*, **67**, 411–415.

Sillén-Tullberg, B. 1988. Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution*, **42**, 293–305.

Sillén-Tullberg, B. 1990. Do predators avoid groups of aposematic prey? An experimental test. *Anim. Behav.*, **40**, 856–860.

Sillén-Tullberg, B. 1992. Does gregariousness reduce attacks on aposematic prey? A reply to Cooper. *Anim. Behav.*, **43,** 165–167.

Sillén-Tullberg, B. 1993. The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. *Evolution*, **47**, 1182–1191.

Sillén-Tullberg, B. & Leimar, O. 1988. The evolution of gregariousness in distasteful insects as a defense against predators. *Am. Nat.*, **132**, 723–734.

Staddon, B. W. 1979. *The Scent Glands of Heteroptera*. London: Academic Press.

Tostowaryk, W. 1972. The effect of prey defense on the functional response of *Podisus modestus* to densities of the sawflies *Neodiprion swainei* and *N. pratti banksianae*. *Can. Entomol.*, **104,** 61–69.

Treisman, M. 1975. Predation and the evolution of gregariousness. I. Models for concealment and evasion. *Anim. Behav.*, **23**, 779–800.

Tullberg, B. S. & Hunter, A. F. 1996. Evolution of larval gregariousness in relation to repellent defences and warning coloration in tree-feeding Macrolepidoptera: a phylogenetic analysis based on independent contrasts. *Biol. J. Linn. Soc.* **57,** 253–276.

Turner, J. R. G. 1975. Communal roosting in relation to warning colour in two heliconidae butterflies (Nymphalidae). *J. Lepid. Soc.*, **29**, 221–226.