Do palatable prey benefit from aposematic neighbors?[1]

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Abstract: Synergistic selection can promote the evolution of aposematism. This requires, however, that palatable prey without signals do not benefit from their aposematic neighbours. In order to test this requirement, we designed an experiment using artificially aposematic mealworm larvae and palatable control larvae. As a predator we used great tits. Birds encountered five types of groups: pure groups of aposematic or palatable prey items and mixed groups of palatable and aposematic items or single palatable and single aposematic preys. Survival of aposematic items both in pure groups and for solitary items was high. Unexpectedly, birds also avoided mixed groups. Survival of palatable prey with aposematic neighbors was significantly higher compared to solitary palatables and to those with palatable neighbors. Thus, our results suggest that the protection through association gained by palatable individuals should be taken into account when evaluating evolutionary consequences of synergism for the evolution of aposematism.
Keywords: aposematism, associational protection, synergism.

Résumé: La sélection synergétique peut favoriser l'évolution de l'aposématisme. Ceci nécessite toutefois qu'une proie comestible et sans signal ne profite pas de la présence de voisins aposématiques. De manière à vérifier la validité de cette exigence, nous avons conçu une expérience utilisant des larves de ver de farine artificiellement aposématiques et des larves témoins comestibles. Comme prédateur, nous nous sommes servis de mésanges charbonnières. Les oiseaux ont été confrontés à cinq types de groupes : des groupes homogènes composées de proies toutes aposématiques ou toutes comestibles, des groupes mixtes comprenant des proies aposématiques et comestibles et enfin des proies solitaires aposématiques ou comestibles. La survie des proies aposématiques a été élevée tant pour les groupes homogènes que pour les individus solitaires. De manière inattendue, les oiseaux ont aussi évité les groupes mixtes. La survie des proies comestibles ayant des voisins aposématiques a été significativement plus élevée que celle des proies comestibles solitaires ou ayant des voisins comestibles. Conséquemment, nos résultats suggèrent que la protection de voisinage dont bénéficient les individus comestibles devrait être prise en considération lorsqu'on évalue les conséquences du synergisme dans l'évolution de l'aposématisme.
Mots-clés: aposématisme, protection du voisinage, synergie.

Introduction

During the nesting period, butterfly larvae are the main food for many species of passerine birds. Some species of butterflies are, however, unpalatable for birds, and aposematic coloration (*e.g.*, black and yellow, orange or red) is common among unpalatable species. Because birds can see colors and easily associate unpalatability with signals, it is likely that warning signals (*i.e.*, aposematic coloration) are effective against bird predators (Gittleman & Harvey, 1980; Roper & Wistow, 1986; Roper & Redston, 1987; Tullrot & Sundberg, 1991). Guilford & Cuthill (1991) suggested that the evolution of aposematism is enhanced by synergistic benefits which are shared by individuals expressing similar warning signals. This requires that the predators show a positive bias in their generalization by most strongly avoiding prey individuals that more strongly express a signal than the prey population on average (Leimar, Enquist & Sillén-Tullberg, 1986, Leimar & Tuomi, 1998). In contrast, a

defensive signal may not be evolutionarily stable if individuals that either do not express the signal, or express it to a lesser extent than the average population, tend to benefit from living in close proximity to aposematic prey. Accordingly, Tuomi & Augner (1993) showed that plant defences may not be evolutionarily stable if palatable plants gain more than unpalatable plants from living close to unpalatable neighbors.

Plant-herbivore studies have demonstrated that palatable plants can sometimes gain an associational protection from their unpalatable neighbors (Tahvanainen & Root, 1972; Atsatt & O´Dowd, 1976; McNaughton, 1978; Hay, 1986; Pfister & Hay, 1988). According to Hjältén, Danell & Lundberg (1993), this essentially depends on whether the herbivores are selective within or between patches of potential host plants. Palatable plants are most likely to gain associational protection if the herbivores avoid unprofitable patches and are relatively unselective within patches (Holt & Kotler, 1987). This situation may also arise in the evolution of aposematic signals since, at least

in aggregated and aposematic prey types, avian predators often leave the rest of the prey group after tasting the first aposematic individual (Gagliardo & Guilford, 1993; Alatalo & Mappes, 1996; Mappes & Alatalo, 1997). We experimentally tested whether palatable prey may benefit from having aposematic neighbors. We designed an experiment using mealworm larvae artificially painted to be aposematic and injected unpalatable and unpainted control larvae. We used experienced predators, great tits, since this is a common situation for most of the prey populations that have evolved warning signals.

Material and methods

Wild captured, adult great tits, *Parus major,* were used as predators of last instar mealworm larvae *Tenebrio mollitor*. The experiments were conducted at the Konnevesi Research Station in central Finland in March 1995. Adult birds were captured around the Research Station with mist-nets. After the experimental trial, birds were banded and then released.

Before the start of an experiment, each bird was acclimatized to the experimental aviary (7.3 m^2). Sunflower seeds were offered in the initial period of 30 minutes and only water was offered after that. Individuals ($n = 4$) that did not feed during the training phase were released before any experimentation. Individual birds were used only once ($n = 18$).

In the experiment, palatable and aposematic prey items were presented on a wooden plate. We made mealworm larvae unpalatable by injecting them with 30 μl of 40% aqueous chloroquine. Before injection we killed mealworm larvae by dropping them into the boiling water for few seconds. We also painted yellow and black dots on the backs of these larvae using children's finger paints. Before the experiment we offered three palatable and three aposematic larvae to tits to ensure that all individuals had experienced both types of prey. The same birds had been used earlier in another experiment where signals (crosses and squares), instead of colors, were attached to artificial prey items (Alatalo & Mappes, 1996).

In the present experiment, we had four blocks to ensure interspersion of items. Each block contained five different "groups" of prey items: (*i*) a solitary palatable prey item, (*ii*) a solitary aposematic prey item, (*iii*) two palatable prey items side by side (*ca* 2 cm apart), (*iv*) two aposematic prey items side by side, and (*v*) one aposematic and one palatable prey item side by side (Figure 1). In the experiment, all five treatments were simultaneously presented to make it possible to compare the survival of solitary and aggregated prey types and to see the change in survival due to the neighbor type. The distance between groups was *ca* 40 cm. Within the four blocks we randomized the order of each of the group of five. To avoid problems caused by possible UV-reflectance of the colors, we used only light bulbs that do not emit UV-light in the aviary (Viitala *et al.*, 1995). The experimental room did not have windows and, thus, natural light did not cause any differences in UV-reflectance. We allowed birds to attack at most 12 prey items, and if a bird did not eat 12 prey items we continued observations for a maximum of 30 minutes. With this experimental setup we could estimate the relative success of palatable/aposematic individuals in relation to the presence of neighbors.

Results and discussion

There was a clear significant difference in relative mortality among the different types of prey (Figure 1; Friedman's ANOVA to related samples, chi-square 67,75, df = 5, $P < 0.001$). Mortality of aposematic prey types was very low (mean ± SE = 7.4 ± 2.0%, $N = 18$) compared to mortality of palatable prey types (63.2 ± 4%). Friedman's ANOVA, while taking into account the dependent data structure, does not allow *a posteriori* comparison of group means. Thus, we used paired Wilcoxon's tests with Bonferroni corrections accounting for the five primary comparisons between treatments.

The test of solitary aposematic against solitary palatable prey ($T = 0$, $p <.0001$) indicates a fitness gain of 66.7% in survival for aposematic individuals (Table I). Since, we used familiar signals, this gain includes mainly (*i*) the protective effects of unpalatability and color signals on the attacked solitary individuals themselves and, in addition, possible (*ii*) synergistic effects among aposematic individuals due to the learning of the predators during the experiment. The tests of solitary aposematic against aposematic prey with an aposematic ($T = 0$, $p = 0.312$) or palatable neighbor ($T= 17.5$, $p = 1.00$) suggest very weak, if any, associational effects (8.3 and -1.4%, respectively; Table I). These associational effects on aposematic prey include group effects which result from (*i*) aggregation as such, and (*ii*) neighbor-type in-aggregations. The latter would indicate synergistic benefit within the group if aposematic prey survive better with an aposematic neighbor than when the neighbor is
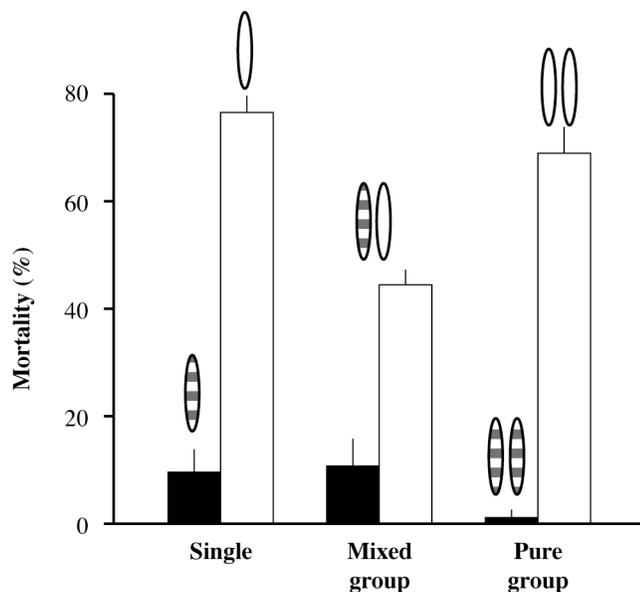

FIGURE 1. The proportion of aposematic (black bars) and palatable (white bars) prey "killed" when they were either solitary or aggregated, in "mixed groups" of an aposematic and a palatable prey or in "pure groups" where both prey individuals were of the same type. Standard errors are indicated above each bar ($n = 18$ predators).

TABLE I. Relative survival of aposematic and palatable prey when presented solitarily or aggregated to predator. Statistical significances of paired Wilcoxon test (the tests for changes are Bonferroni-corrected, see text): $ns = P > 0.1$, $a = P < 0.10$, $* = P < 0.05$, $** = P < 0.01$, $*** = P < 0.001$

Prey type	Survival of solitary prey (%)	Change in survival due to the neighbor of type:		Benefit from having Aposematic as a neighbor instead of palatable
		Aposematic	Palatable	
Aposematic	90.3	8.3^{ns}	-1.4^{ns}	9.7^{a}
Palatable	23.6	32.0^{***}	7.6^{ns}	24.4^{**}
Aposematic - Palatable	66.7^{***}			-14.7^{a}

palatable. For aposematic prey with an aposematic instead of a palatable neighbor ($T = 3.5$, $p = 0.078$), our estimate of such synergistic benefits was an increase in survival by 9.7%. This is a relatively small benefit when compared to the survival of solitary aposematic larvae (90.3%; Table I).

We expect that the above synergistic benefit within groups would have been stronger and the survival of solitary larvae lower if we had used totally unexperienced, naive predators. In an earlier experiment with novel signals (Alatalo & Mappes, 1996), the survival of solitary aposematic prey increased during successive trials (Trial I: 63.4%; II: 83.0%; III: 86.5%) and the difference between aggregated and solitary aposematic prey items decreased (I: 17.0%; II: 4.5%; III: 4.5%). Our present estimate of the change in survival due to an aposematic neighbor (8.3%) is thus comparable to the last trial of this earlier experiment.

Because our birds were experienced adults, we intuitively expected that they would easily spot and attack palatable larvae with aposematic neighbors. In contrast, the test of solitary palatable against palatable prey with an aposematic neighbor ($T = 2.5$, $P = 0.002$) indicated unexpectedly high associational effects (32.0%; Table I). Palatable prey thus survived better when they were placed next to an aposematic neighbor as compared to solitary larvae. The corresponding effects (7.6%) were not statistically significant when the neighbor was palatable ($T = 30$, $P = 1.00$). Thus the protective effects resulted from neighbor-type rather than from aggregation as such. The estimated associational gain for palatable larvae of having an aposematic instead of a palatable neighbor was 24.4% ($T = 23.5$, $p = 0.009$). These effects presumably arise from the foraging and prey handling behavior of the tits. They typically pick up the attacked prey and move to a suitable place for handling and consuming larger prey items. If the prey is unpalatable, they seem less likely to return to the sampled prey group, thus enhancing survival of palatable larvae associated with aposematic neighbors. Moreover, once the predators are familiar with the warning signals, the signalling prey will be protected from a distance. Thus, associational gain for palatable prey with an aposematic neighbor may also arise from predators not approaching the group at all.

A comparison of the observed synergistic benefits of aposematic prey (9.7%) to the associational gain of palatable larvae (24.4%) reveals that the balance is negative (-14.7%; Table I). These estimates are comparable to the game theory model of Tuomi & Augner (1993; Equation 3) suggesting that the balance should be positive if synergism

is to enhance the evolution of aposematism as suggested by Guilford (1985) and Guilford & Cuthill (1991). The negative balance would counteract the evolution of aposematism (Queller, 1984; Tuomi & Augner, 1993), but in our case, due to benefits among solitary individuals, the aposematic prey type would still have a higher survival rate than the palatable one (66.7-14.7 = 52.0%). We therefore conclude that (i) associational protection gained by palatable larvae from aposematic neighbors may outweigh the possible synergistic benefit within a group and (ii) that aposematism is favored either by benefits solitary larvae themselves directly gain from their own bad taste, and/or by synergistic benefits for color that are not related to physical association of the larvae. The last alternative appears particularly relevant here, since we used familiar signals with experienced predators.

The present fitness effects (Table I) are only suggestive estimates which may vary considerably depending on the visual and learning capabilities of the predators and their earlier experiences, prey types (degree of unpalatability, signal type and strength, degree of crypsis), and the complexity and heterogeneity of the environment. In spite of this caveat, it is important to recognize the possibility that palatable prey types can gain associational protection from aposematic neighbors. Even strong synergism may fail to enhance the evolution of aposematism if it is nullified by equally strong or even stronger associational profits of palatable types.

Acknowledgements

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