Effects of novelty and gregariousness in survival of aposematic prey

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We examined the reactions of captive wild great tits to novel unpalatable prey with (1) a traditional aposematic signal (black and yellow), (2) a novel signal (light pink), and (3) a control signal (brown). Prey were offered either singly or in groups to see whether novel signals with fewer possibilities for synergistic benefits are more dependent on grouping than are the traditional signals. Indeed, responses of birds toward unpalatable prey depended significantly on spatial distribution of prey (grouping versus solitary) and the type of the signal. Birds avoided more the traditional black and yellow signals than pink signals in both experimental set ups, but both of these prey items survived better in aggregation than solitary. The success of traditional signals may demonstrate the importance of synergistic selection across species in the evolution of warning coloration (i.e., Müllerian mimicry). Unpalatable prey individuals benefit strongly from using similar color patterns. Our results suggest that aggregation may be important for the evolution of novel signals in particular, even if a synergistic selection component is also present. Key words: Aposematism, great tit, gregariousness, novel signal, Parus major, traditional signal.

Unpalatable or otherwise unprofitable prey species have often evolved a conspicuous warning coloration or exhibit special distinctive signals (Cott, 1940; Edmunds, 1974; Guilford et al., 1987). Such a combination of traits is called aposematism. Although the function of aposematism in allowing the predators to associate noxiousness with the signal is well tested (e.g., Gittleman and Harvey, 1986; Roper and Redston, 1987; Roper and Wistow, 1986; Tullrot and Sundberg, 1991), evolutionary pathways from cryptic palatability to conspicuous unpalatability are more problematic to explore (Endler, 1991).

The relative role of individual benefits, kin benefits, and synergistic benefits in the evolution of conspicuously colored and unpalatable prey have been widely discussed (Endler, 1988; Guilford, 1988, 1990a; Leimar et al., 1986; Sillén-Tullberg and Leimar, 1988). It has been experimentally documented that gregariousness enhances discriminative aversion learning in aposematic prey (Gagliardo and Guilford, 1993), supporting the idea that kin selection might be essential in the origin of aposematism (Fisher, 1930; Guilford, 1988, 1988; Harvey and Paxton, 1981; Harvey et al., 1982). On the other hand, in many cases aposematic prey seem to also manage well solitarily, predators may leave the signaling prey unkillled after some initial handling, thus supporting straightforward individual selection (Edmunds, 1974; Järvi et al., 1982; Sillén-Tullberg, 1985a; Wiklund and Järvi, 1982).

The idea of synergistic selection is similar to the "green beard effect" (Dawkins, 1976). Unpalatable individuals may benefit by using similar warning colors, or any other signals, that other individuals of the same or other species have used (Guilford, 1990a,b; Maynard Smith, 1989). Then, prey individuals with traditional color patterns such as black and yellow or orange stripes should be more protected against predators than those exhibiting any new signals, which is to be expected from the traditional Müllerian mimicry operating across the species level.

To study the ancestral origin of warning signals, it would not suffice to use naive predators because avoidance of warning colors is also likely to have a genetic basis (Gehlbach, 1972; Rubinoff and Kropach, 1970; Schuler and Hesse, 1985; Smith, 1975, 1977). Thus, we did not use naive birds in this experiment, since we were tracking the situation when warning coloration appears in a new species in the world where many other unpalatable prey already have warning signals. This is the more common situation for most of the prey populations that have evolved warning signals, but obviously the situation would have been different in the ancestral history when warning coloration appeared for the first time. This paper examines the reactions of captive wild great tits to novel unpalatable prey with (1) a traditional signal (black/yellow), (2) a novel signal (light pink), and (3) a control signal (brown). The prey items were offered either in groups or solitarily to allow us to explore the benefits of signaling in relation to signal type and the spatial distribution of the prey.

METHODS
Adult great tits, Parus major, were used as predators, last-instar mealworm larvae, Tenebrio molitor, painted and artificially made distasteful, were chosen as prey. The experiments were done at Konnevesi research station in Central Finland. In this area meal worm larvae are not available in nature, and thus birds had no experience of the prey items, which are naturally tasteful to birds and not aposematic. We captured and ringed adult birds during the late nestling period in June 1994. After the approximate 2-h experimental trial, birds were released back to their nests. In each nest only one of the parents was used at a time, and the short absence of the parents had no effects on survival of the offspring.

Before the start of an experiment, we allowed a bird to use the experimental aviary (7.5 m²) for 90 min. Sunflower seeds were offered during the first 30 min of this habitation period, and only water was offered after that. Those that did not feed during the training phase were released before any experimentation. We used each bird only once. We made mealworm larvae unpalatable by injecting 0.03 ml Tabasco sauce inside them. We randomly divided the injected larvae into three groups and painted five dots on their backs with non-poisonous children's finger paints using either black and yel-
low (B/Y), light pink (P), or brown control (C). We checked in a blind taste test (n = 5 colleagues) that the colors were not separable according to their taste. Thus, B/Y represented novel prey that uses a commonly existing warning color combination, and P represented novel prey with a new or at least an uncommon signal for warning. There are many unprofitable prey types with black and yellow stripes in the habitats where the birds were captured (e.g., wasps, bees, bumblebees and caterpillars such as Parnassius brassicae, Phalera bucephala, Lecanobia oleracea). C was novel prey that did not warn its unpalatability, as brown is the original color of the larvae. To avoid the problems caused by possible UV-reflectance of the colors, we used in the aviary only light bulbs that do not emit UV-light (see Viitala et al., 1995) and no natural light was present.

We presented the artificially unpalatable prey items either singly or in groups of four individuals of each type (black/yellow, pink, and control) on a small, white plastic tray (7 cm diam). Thus, all prey types were highly contrasted with the background, and the signals, not the conspicuousness, were likely to be tested (cf. Sillén-Tullberg, 1985b). When prey items were offered in aggregations, four prey individuals of each type were offered at the start of the experiment in three separate trays side by side (in a triangle). When prey were presented singly, one prey of each type was placed on separate trays again side by side. To mimic the situation in which birds find the prey items dispersed during their search of food, every 5 min a new tray with three single prey items was placed in the test room approximately 1.5 m from where the previous one had been. Each rearrangement of the solitary prey was done while the lights were turned off, and birds in aggregated treatments were disturbed similarly. Thus, a total four prey items of each type were presented during the experiment. The experiment started when the bird began to search the food. The experiment lasted 30 min. During each trial, we recorded bird behavior including attacks and handling of prey. After the experiment, we checked how many prey items had been eaten or seriously damaged (and thus considered as dead) during encounters with predators. The experimental setup was designed to be particularly effective in the sense that in both treatments (aggregated/dispersed), birds were given a choice of the three prey types. The simultaneous presentation of the three prey types allowed us to see the preferences of birds without any confounding effects caused by variability in sequence. Altogether 48 birds were used, equally divided to the 2 treatments (24 in aggregation and 24 in solitary set up).

In a second "control" experiment, dead mealworm larvae, with no distastefulness nor warning coloration, were offered either singly or aggregated to another set of 10 birds. The setup was identical to the first experiment, and its purpose was twofold: to show that birds would readily feed on many of the 12 larvae if they did not contain unpalatable substances, and to check whether aggregation without unpalatability might have affected the number of larvae eaten in our two types of treatments.

RESULTS

In the analyses we have only applied the number of "killed" prey as the dependent variable, since most of the attacked prey were at least partly eaten or seriously damaged by the hammering type of prey handling typically used by great tits. Adding up all the observations, there was a slight tendency for the probability of being killed to be smaller for B/Y larvae (76.9%, n = 52 attacked items) than for P larvae (89.9%, n = 69) or C larvae (89.8%, n = 127).

Two-way analysis of variance with repeated measures over the three color types shows a significant main effect due to the type of the treatment (aggregated versus dispersed; F₁,₄₄ = 14.92, p < .001). There was a significant main effect for color (within-subject factor, Pillais F₁,₄₄ = 51.17, p < .001; Figure 1). The repeated-measures ANOVA, while taking account of the dependent data structure, did not allow a posteriori comparison of means. However, in this case we are clearly expecting ranked order in the use of different color types, so that traditional B/Y signaling prey would be used least and control prey most frequently. Accordingly we can, a priori, compare two sets of means by a paired t test; B/Y against P and P against C. In aggregations, the use of B/Y did not differ from P (t = −.98, 23 df, p = .357), but C was used much more than P prey (t = 4.42, 23 df, p < .001; Figure 1). Among solitary presentations, however, B/Y prey were clearly less used than P (t = −2.80, 23 df, p = .010), and the difference between P and C was again highly significant (t = 2.91, 23 df, p = .008). Using the total number of attacked prey items, including the few larvae left undamaged, gave nearly identical test results.

Both B/Y and P mealworm larvae suffered significantly higher mortality in the dispersed treatment than in the aggregated one (t = −3.18, 44 df, p = .003 and t = −3.85, 44 df, p < .001, respectively). However, mortality of controls did not differ significantly between two aggregation levels (t = −1.35, 44 df, p = .185). Thus, both the traditional and novel...
signals benefited through a reduced number of harmful attacks when the prey were aggregated. Consequently, the total number of prey killed was also lower among the aggregated treatments (mean = 5.41, SD = 1.47, n = 24) than among the dispersed treatments (mean = 5.58, SD = 2.32, n = 24, t = 0.86, p < .001).

In the control tests, where all prey items were palatable and unpainted, most of the 12 larvae were killed and consumed in both the aggregated and the dispersed treatments (11.2 ± 0.84, n = 5 and 9.6 ± 2.30, n = 5, respectively). Thus, there was no significant difference between the treatments (t = 1.46, df, p = .182). If anything, the tendency was for more of the aggregated larvae to be consumed, indicating that aggregation alone did not have any benefits for palatable prey.

**DISCUSSION**

The results of our experiment show that a traditional signal is more effective than a novel signal and in aggregations both of these prey types manage to survive better than in solitary presentations. How do these results relate to the discussion about individual, kin, and synergistic benefits in the evolution of aposematism?

In our experiment, pure individual selection based on immediate benefits for the attacked prey (Edmunds, 1974; Järvi et al., 1982; Sillén-Tullberg and Bryant, 1983; Wiklund and Järvi, 1982) was not important: birds ate or seriously damaged vival by 62% and prey with a traditional signal by 57% by unpalatable prey (Guilford, 1988).

Aggregation benefit appears as the difference in survival chances of dispersed prey with novel signals in comparison to controls. This novel versus control difference in the survival chances of dispersed prey with novel signals when prey are dispersed. The dispersed B/Y prey had 38% lower mortality than the P. The corresponding advantage for the traditional signal in aggregation was 30%.

Synergistic selection at a within-species level appears as a difference in the survival chances of dispersed prey with novel signals in comparison to controls. This novel versus control reduction cannot be attributed to aggregation benefit or Müllerian mimicry. The P prey had a 26% lower mortality risk than C when prey were presented solitarily (Figure 1).

Synergistic selection across species level (Müllerian mimicry) appears as the difference in the survival chances of prey with traditional signals in comparison to prey with novel signals when prey are dispersed. The dispersed B/Y prey had 38% lower mortality than the P. The corresponding advantage for the traditional signal in aggregation was 30%.

Aggregation benefit appears as the difference in survival chances of aggregated prey items in comparison to dispersed prey. Unpalatable prey with a novel signal enhanced their survival by 62% and prey with a traditional signal by 57% by being in a group in comparison to their solitary counterparts. Kin selection is classically used to explain why gregariousness is so common among aposematic insects (Fisher, 1950; Guilford, 1985; Harvey, 1983; Harvey and Greenwood, 1978; Harvey and Paxton, 1981; Harvey et al., 1982; Malcolm, 1986), and most gregarious insects do live in kin groups. Kin selection, however, is not necessarily needed to explain evolution of aposematism in aggregations (see also Sillén-Tullberg, 1988). It is the aggregation itself that helps the aposematism prey types predators learning the group after tasting the first unpalatable prey (Guilford, 1988).

The above numerical exercise, while illustrating the different selection types and their general magnitudes, should not be taken too literally. The level of prey dispersion is a continuously variable feature. While our solitary test did not have a high level of spatial distribution, we increased dispersion by presenting the items spaced in time. An ordinary great tit would move considerable distances during the 5-min intervals while searching for their food. Furthermore, although light pink color is not used as a warning signal by any known aposematic prey of the great tits in Finland, we cannot totally exclude the possibility that it resembles other warning signals in a way that enhances synergistic selection through Müllerian mimicry. It may be that the mere contrasted pattern created by light pink spots has such an effect. The light pink color used in the experiment is, however, more novel than the black/yellow as a warning signal. On the other hand, it could also be that black and yellow signals are more effective as signals per se (Endler, 1988, Guilford, 1990a), which is a possibility that is hard to separate experimentally from inherited avoidance.

Our results suggest that aggregation may be important for the evolution of novel signals in particular, even if a synergistic selection component is also present. In fact, even traditional signals benefited strongly from grouping; in spite of the fact that birds did avoid them, probably because of their previous experience with similar warning colors. Avoidance may even have a genetic basis as shown in earlier studies (Gehlbach, 1972; Rubinoff and Kropach, 1970; Schuler and Hesse, 1985; Smith, 1975, 1977). When unpalatability occurred without signal, prey survival did not differ between groups. We must, however, keep in mind that in the present experiment, predators were presented with a simultaneous choice, whereas in nature they will usually have to rely on sequential encounters of different prey items. Thus, the experiment was effective in showing any differences in choosing or avoiding particular prey types.

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