

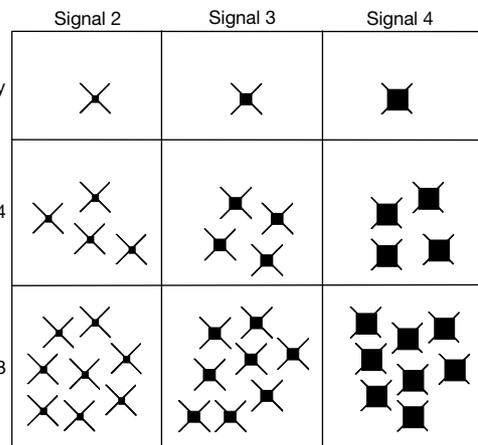
## Multiple benefits of gregariousness cover detectability costs in aposematic aggregations

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Understanding the early evolution of aposematic (warning) coloration has been a challenge for scientists, as a new conspicuous morph in a population of cryptic insects would have a high predation risk and would probably die out before local predators learnt to avoid it<sup>1–4</sup>. Fisher<sup>5</sup> presented the idea of aggregation benefit through the survival of related individuals; however, his theory has been strongly debated<sup>6–8</sup> as the mechanisms that favour grouping have never been explored experimentally with the incorporation of detectability costs. Here we create a comprehensive ‘novel world’ experiment with the great tit (*Parus major*) as a predator to explore simultaneously the predation-related benefits and costs for aposematic aggregated prey, manipulating both group size and signal strength. Our results show that grouping would have been highly beneficial for the first aposematic prey individuals surrounded by naive predators, because (1) detectability risk increased only asymptotically with group size; (2) additional detectability costs due to conspicuous signals were marginal in groups; (3) even naive predators deserted the group after detecting unpalatability (dilution effect); and (4) avoidance learning of signal was faster in groups. None of these mechanisms require kin selection.

In the first experiment we tested how signal strength and group size affects the risk of prey detection. We used the ‘novel world’ method<sup>6,9</sup> because it uses a fundamental property of warning signals, conspicuousness, but presents the predators with signals not found in their natural environment. As prey, we used pieces of almond glued between two pieces of paper that had the signal printed on them. The birds were simultaneously presented with nine different palatable prey assemblages—combinations of three group sizes (1, 4 or 8 prey items) and three signals (a cross symbol with a square set at the centre: the size of the square varied according to the signal number, that is, small (signal 2) to large (signal 4); see Fig. 1). The background on the aviary floor consisted of white paper sheets with the cryptic signal (signal 1: a cross symbol without a square) printed on them. Each prey assemblage was presented four times in random locations on the background. Increasing group size caused an increase in the number of those assemblages that were attacked by the birds ( $F = 84.32$ , degrees of freedom (d.f.) = 2;  $P < 0.001$ ), owing to an increase in prey detectability. Prey groups had higher detectability risk than solitary prey, but this risk did not increase in direct proportion to group size (this effect is sometimes called concealment<sup>10</sup> or avoidance effect<sup>11</sup>) (Fig. 2).



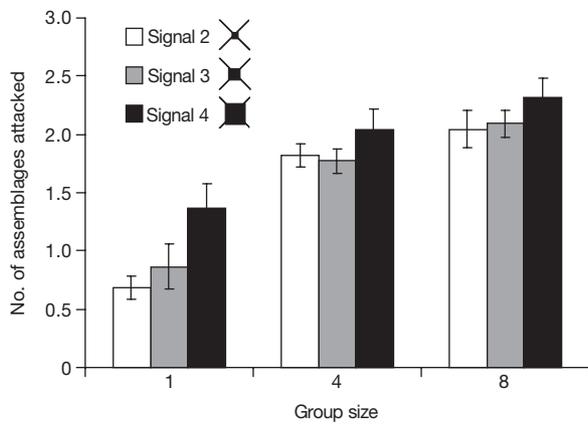
**Figure 1** Prey assemblages used in the detectability experiment. Each assemblage was presented in the aviary in four replicates, and thus there were 36 assemblages in total.

Increasing signal strength caused an increase in the number of those assemblages that were attacked by the birds ( $F = 9.91$ , d.f. = 2,  $P < 0.001$ ). For solitary prey, a strong signal (signal 4) increased the detectability of the prey twofold (100%) compared with signal 2 (Fig. 2;  $t$ -test between solitary signal 2 and 4,  $t = 3.80$ , d.f. = 64,  $P = 0.001$  after Bonferroni correction), whereas in groups of four or eight prey items the increase in the detectability of the group caused by the strong signal was only 12.5% and 13.3%, respectively (Fig. 2; group of 4:  $t = 1.34$ , d.f. = 64,  $P = 0.561$ ; group of 8:  $t = 1.69$ , d.f. = 64,  $P = 0.288$  after Bonferroni correction). Thus, signal strength caused little additional increase in detectability when in a group, suggesting that the cost of a strong signal could be smaller for aggregations than for solitary prey. Previously, the effect of the strength of an aposematic signal on the detectability of prey has been assumed to be similar for both a small group (under 20 individuals) and for solitary prey<sup>10,12</sup>.

If the increased detectability of a group compared with solitary prey does not translate into increased mortality risk per individual, then grouping is always more favourable than a solitary lifestyle. A decreased per capita mortality risk can be produced by a dilution effect, which means that when a predator cannot eat the whole group, an individual's chance of being eaten is smaller in a group than when it is solitary<sup>10,11,13</sup>. To show the effect of dilution, we used the detectability data (Fig. 2) to calculate estimates of relative mortality for average prey individuals in different group sizes in situations where the predator tastes a certain number of prey items from a group (Table 1). This simulates different levels of predator satiation<sup>12</sup>, for example, if the prey is very unpalatable the predator may leave the group after tasting only one individual. Naturally, the real group sizes favoured by selection are dependent on the predator and prey in question, but the general idea is that prey individuals with strong signals benefit most from gregariousness, because their detectability is increased only slightly by grouping (Table 1).

In the second experiment, we performed learning tests with six groups of birds in a factorial design with two signal levels (signal 2 (weak) and signal 4 (strong)) and three prey group sizes. Each bird was presented with solitary, palatable cryptic items (the symbol on the prey was similar to the background symbol) together with unpalatable prey items carrying a signal of only one type. Solitary cryptic prey were palatable to mimic a situation where a distasteful aposematic morph evolves in an environment where most of the cryptic species are palatable; there is thus a reward of learning to avoid the aposematic prey. The tests were carried out in five consecutive days for each bird, and numbers of cryptic and aposematic prey eaten at the trials were recorded. Survival of unpalatable prey with the weak signal (signal 2) increased with group size (Fig. 3a;  $F_{(2,20)} = 3.74$ ,  $P = 0.042$ ). The birds did not learn to avoid

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**Figure 2** Detectability risk of each prey assemblage as the number of attacked prey assemblages in the detectability experiment. Error bars indicate s.e.m.

unpalatable prey, as trial had no effect on survival ( $F_{(4,17)} = 2.10, P = 0.126$ ). There was no interaction between learning (trial) and group size ( $F_{(8,36)} = 0.44, P = 0.890$ ). Thus, the birds did not learn to use either the signal (no learning in solitary treatment) or gregariousness alone (no learning in group treatments) as a sign of unpalatability. As with the weaker signal, survival of the prey with signal 4 increased with group size (Fig. 3b;  $F_{(2,22)} = 9.10, P = 0.001$ ). However, the birds learnt to avoid the aposematic prey, which increased the survival of the aposematic prey in the later trials ( $F_{(4,19)} = 17.95, P < 0.001$ ). Learning was faster with bigger groups of prey items (Fig. 3b; interaction between learning and group size:  $F_{(8,40)} = 2.18, P = 0.050$ ).

We combined the two experiments to compare the observed mortality of unpalatable prey in the learning experiment with the mortality that would be expected if the birds ate the aposematic prey according to their detectability. From the detectability risks of different prey assemblages (Fig. 2), we calculated expected mortality values (horizontal lines in Fig. 3) for the learning experiment, assuming that all the prey items would be eaten from a group once it was found. The birds ate distasteful prey items that displayed signal 2 according to their detectability only when the prey were solitary (Fig. 3a). When the prey items were gregarious, they suffered lower mortality than expected on the basis of their detectability. The difference between the expected and observed mortality of prey increased with increasing group size (Fig. 3a). The enhanced survival of aggregated unpalatable prey with a weak signal was probably due to a dilution effect, as a result of the birds leaving the group after eating one or a few prey items and detecting unpalatability. Dilution is beneficial only if the encounter probability of a group is smaller than that of an equal number of solitary prey

**Table 1** Effect of dilution on per capita mortality risks for prey individuals in different group sizes

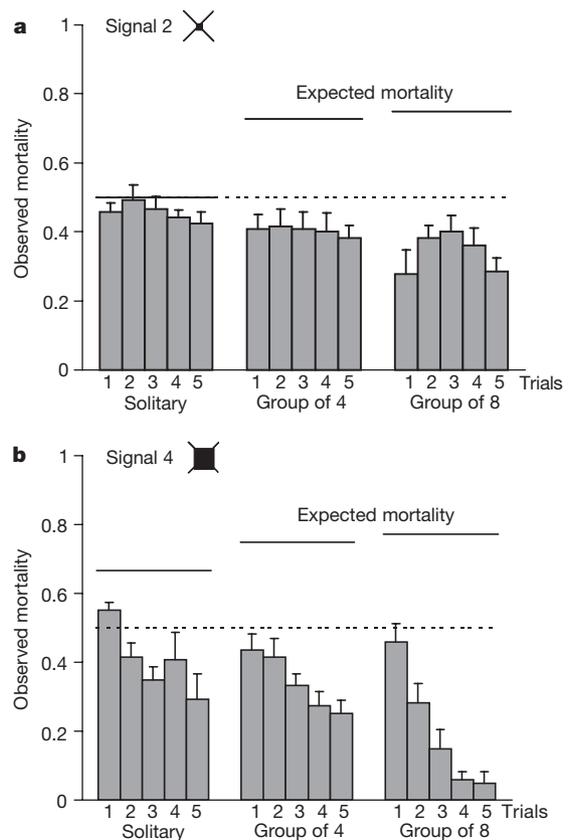
Signal	Group size	Average mortality risk for an individual				
		1 eaten	2 eaten	3 eaten	4 eaten	8 eaten
Signal 2	4	0.67	1.33	2	2.67	—
	8	0.38	0.75	1.13	1.50	3
Signal 3	4	0.51	1.03	1.54	2.05	—
	8	0.30	0.61	0.91	1.21	2.42
Signal 4	4	0.38	0.75	1.13	1.50	—
	8	0.21	0.43	0.64	0.85	1.70

Risks were calculated relative to the detectability of the similar solitary prey (defined as 1) within each signal. Thus, comparisons should be made only between group sizes within a signal, as the values for different signals are not comparable. The original values for the groups are the attack numbers from the detectability experiment (relative to the detectability of the solitary item within each signal), which are divided by the number of prey in the group to get a theoretical risk for one individual and multiplied by the number of prey eaten in each case. For example, even if four items are eaten from a group of eight with signal 4, it is better for the individuals with this signal to live in a group of eight than solitary, because the mortality risk per average individual is lower in the group (0.85) than solitary (1.0). Numbers less than 1 indicate when it is advantageous for prey to live in a group rather than solitary.

items<sup>11</sup>. This was the case here because in the detectability experiment the detectability risk of a group did not increase linearly with group size. Because the dilution effect is based on proximity cues and works before the predator has learnt to avoid a group on the basis of the signal, it can also help cryptic unpalatable prey that live gregariously. Signal 2 in these experiments was effectively cryptic, as it did not differ from signal 1 in a visibility test with solitary prey<sup>9</sup>, and it did not cause avoidance learning in the birds even after repeated experience.

Aposematic prey items with a strong signal (signal 4) were eaten less than predicted from their detectability in all group sizes, even in the first trial (Fig. 3b). This might be due to a neophobic reaction towards unfamiliar prey<sup>14</sup>, because to birds the prey item with a strong signal might be visually more different from the white training baits than the cryptic prey item. The expected mortality was based on the detectability test, in which the birds had previous experience with all the signal types and did not show any initial avoidance. In aggregations, the difference between expected and observed mortality in the first trial was too large to be explained only by neophobia (compare the first trial in solitary treatment with the first trial in group treatments, Fig. 3b). This suggests that dilution was also functioning with the stronger signal.

We demonstrate that gregariousness has more benefits for aposematic prey than just faster avoidance learning<sup>6,15</sup> and possible kin benefits<sup>5</sup>. For the more conspicuous signal, gregariousness provided many advantages that lead to the better survival of the signal



**Figure 3** Observed and expected mortality of unpalatable prey as the proportion of unpalatable items from all prey that were eaten. Bars represent observed mortality (with standard error) for each treatment in the five trials. The dashed line represents equal mortality for palatable cryptic and unpalatable aposematic prey. The three solid horizontal lines indicate expected mortality for each group size estimated from the detectability risks in Fig. 2. Expected mortality was calculated relative to the detectability of solitary prey displaying signal 2, assuming that all prey items in a group were eaten once it was found. **a**, Signal 2. **b**, Signal 4.

carriers. Avoidance learning, which was faster with the larger aggregation, required a strong signal, but the signal had no initial cost when the prey were gregarious. The signal increased detectability only slightly and detectability costs resulting from both the signal and aggregation were counterbalanced by the dilution effect, by decreased per capita encounter probability and possibly by neophobia. Notably, the dilution effect increased the survival of aggregated unpalatable prey even without a strong warning signal. Thus, unpalatability alone could select for grouping under the influence of individual selection, and in groups the evolution of a stronger signal would be favoured by synergistic selection<sup>6,16</sup>, which affects individuals of the same phenotype regardless of their ancestral relatedness. Alternative explanations for the initial evolution of warning coloration have been proposed<sup>17</sup>, such as random drift, neophobia<sup>14</sup>, evolution through individual selection when prey are by some means able to survive an attack<sup>18–20</sup>, or the coloration being cryptic from a distance but aposematic when the predator is close<sup>21</sup>. Most of these hypotheses are not mutually exclusive, and different mechanisms may have been important in distinct areas or with different species, thus we do not claim that gregariousness is a prerequisite for the evolution of warning signals. However, given the dilution effect, the small detectability costs of signals in groups and the enhanced learning of strong signals in groups, it seems that gregariousness of unpalatable prey might have enabled the initial appearance of aposematism, and grouping may assist in the survival of established aposematic prey whenever the prey encounter naive predators. □

## Methods

### Predators and prey

Wild great tits were caught in mist nets around Konnevesi Research Station where the experiments were carried out from January to May 1997 (general methods as in ref. 9). Each bird was trained to open similar paper prey items to the ones that were eventually used in the experiments, but during the training the prey items had no signal. After the experiment we released great tits to the area where they were caught.

### Detectability experiment

All of the prey items were palatable as the objective was to find out how group size and signal conspicuousness affect the number of prey attacked owing to detectability differences. Signal 1 (the background signal) was not used because in a separate visibility test with solitary prey items, signal 1 and signal 2 did not differ significantly in their conspicuousness to the great tits (result reported in ref. 9). Before the detectability test, the birds ( $n = 11$ ) were given palatable prey items that displayed all of the signals used so as to avoid neophobic reactions towards any of the signals. Eating or touching the prey item was taken as an indication that the bird had seen the prey, as the birds had no reason to avoid any of the prey types. The trial continued until the bird had attacked 20 prey assemblages, but only the first 15 assemblages were included in the final analysis to avoid the risk that prey depletion during feeding would bias the detectability estimation. The experiment was repeated the next day and the mean values from two trials were used in further calculations, because the trials gave similar results. The data were analysed with a two-way analysis of variance (ANOVA) with main effects (SPSS for Windows version 7.0).

### Learning experiment

We tested selection pressures on evolving aposematic prey by using palatable cryptic prey items with signal 1 together with unpalatable items with either signal 2 or signal 4. Signal 3 was not included because it did not differ much from signal 2 (see Fig. 2). Each bird was randomly assigned to one of the six treatment groups (two signal strengths  $\times$  three group sizes), so that every treatment had 7–9 birds (total  $n = 48$  birds).

In each treatment half of the prey items in the aviary were cryptic, palatable and solitary, whereas the other half were aposematic (unpalatable and displaying a signal) and were placed either solitarily, in groups of four or in groups of eight. The number of prey items was always the same: 24 palatable and 24 distasteful items in the aviary. The birds were allowed to taste 15 prey items in each of the five trials. The number of unpalatable prey items eaten in a trial was used as a dependent variable in repeated measures ANOVA (SPSS for Windows version 7.0), with trial as the within-subject factor (corresponding to learning) and group size as a between-subject factor. Separate tests were performed for the two signals. The data for solitary treatment, which serves as the control here, were obtained from another experiment that was performed at the same time<sup>9</sup>.

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