



## The evolution of aggregation in profitable and unprofitable prey

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The relationship between gregariousness and warning signals in defended species has been the subject of much debate. While previous researchers have found that predators learn to avoid conspicuous unpalatable prey faster when they are in aggregations, they have not presented solitary and aggregated unprofitable prey to predators simultaneously to directly compare their survivorship. Furthermore, no experiment has investigated the influence of profitable prey configuration on predator response to aggregation in unprofitable prey. Here we investigated the response of human, *Homo sapiens*, predators to profitable and unprofitable prey in a computer version of the 'novel world' experiments that addressed these issues. When all prey types were cryptic and all profitable prey were solitary, then aggregated unprofitable prey had higher per capita survivorship than solitary unprofitable prey. In this case, aggregation was the sole reliable distinguishing characteristic of unprofitability. By contrast, when profitable and unprofitable prey could be distinguished in terms of their conspicuousness, then aggregation did not confer a benefit to unprofitable prey. When profitable prey were simultaneously presented in solitary and aggregated configurations, aggregated profitable prey consistently had a lower per capita survivorship than solitary profitable prey, regardless of the configuration of unprofitable prey. We conclude that while aggregation can serve as a signal of unprofitability, it may not be required for the evolution of warning signals. Instead, we suggest that the common association between aggregation and distastefulness may primarily arise for a 'negative' reason: the vulnerability of aggregated palatable prey.

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The conditions under which aposematism, the conspicuous coloration of unpalatable or otherwise defended prey, could evolve have long been a topic of speculation (Wallace 1867; Poulton 1890). A perceived roadblock to the initial establishment of rare, aposematic mutants is the intense predation to which they would be subjected by naïve predators. Conspicuous prey, albeit defended, are much more likely to be seen by predators, and if predators are unaware of their defence (and do not show neophobia), then such prey are more likely to be attacked on encounter (Gittleman & Harvey 1980). This means that rare conspicuous mutants of defended prey should, on average, be attacked more frequently than their cryptic conspecifics.

A possible solution to this problem, first suggested by Fisher (1930, page 159), is that gregariousness could facilitate the evolution of distastefulness (and hence aposematism). Thus, if prey are warningly coloured and

aggregated, then an attack on one individual by a naïve predator could lead to subsequent avoidance of others in the group, often relatives, that share the same trait (this proposal was the initial inspiration for Hamilton's (1963) theory of kin selection (Hamilton 1996)).

A number of researchers have investigated the relationship between aggregation and aposematism. Some have found evidence that avoidance learning is faster when warningly coloured prey are aggregated (Gittleman & Harvey 1980; Gagliardo & Guilford 1993; Alatalo & Mappes 1996; Riipi et al. 2001), although some of these conclusions have been challenged (Tullberg et al. 2000). Others have argued that aposematic prey in aggregations present a more intense signal to their predators (Gamberale & Tullberg 1996, 1998; Sherratt 2002). Furthermore, a dilution effect may improve the survivorship of grouped aposematic prey, especially if the level of detectability of aggregations does not increase linearly with group size or increasing signal conspicuousness (Bertram 1978; Sillén-Tullberg 1988; Riipi et al. 2001).

One factor complicating experimental investigation of this phenomenon is the prevalence of aposematic

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organisms in nature. Current predators are not naïve in the evolutionary sense, and experiments with natural predators are therefore potentially confounded by pre-established responses to defended conspicuous prey. To address this difficulty, Alatalo & Mappes (1996) devised a series of 'novel world' experiments, using wild-caught great tits, *Parus major*, as predators. The great tits foraged in an arena where the floor was covered in one of two symbols, either crosses or black squares. Distributed on this background were palatable and unpalatable prey items labelled with one of these two symbols. A prey item was 'cryptic' if the symbol it carried was similar in appearance to the background symbol, whereas unpalatable prey items were classed as 'aposematic' if they carried a different (effectively conspicuous) symbol. Through this method, prey items presented signals that bird predators could learn to associate with (un)palatability, but that were unlike signals that the predators would have experienced in nature.

In this study we replicated Alatalo & Mappes' 'novel world' experimental conditions in a computer environment, using humans, *Homo sapiens*, as predators (see Tucker & Allen 1993; Glanville & Allen 1997 for parallel approaches using humans to address questions relating to crypsis). We have recently investigated questions about the initial evolution of aposematism with a similar system of computer experiments (Sherratt & Beatty 2003), and wished to ask further questions about aposematism and aggregation by expanding on Alatalo & Mappes' imaginative approach. To compare our results with humans to those found with great tits, we first repeated Alatalo & Mappes' 'initial origin' experiments. If their results were truly robust, we might expect the same general outcome with another species, albeit one with a high potential for learning and strategizing.

We then used this experimental format to test several important questions about the relationship between aggregation, conspicuousness and profitability that have so far not been addressed. First, we wished to investigate the effects of profitable prey configuration on the adaptiveness of aggregation in unprofitable prey. We believe that this issue is important to consider because it is quite possible that the form of a signal that is most advantageous to unprofitable prey (e.g. aggregation and/or conspicuousness) is to some degree dependent on the traits shown by profitable prey (and vice versa, Sherratt & Beatty 2003). Therefore, we designed experiments to address the following questions. (1) Will aggregation evolve in cryptic unprofitable prey if it reliably indicates unprofitability? (2) Will aggregation evolve in conspicuous unprofitable prey if it is a reliable, yet superfluous, indicator of unprofitability? (3) Will aggregation evolve in cryptic unprofitable prey if cryptic profitable prey are also aggregated? Finally, to understand why unprofitability is associated with aggregation, it is clearly helpful to elucidate how readily aggregation evolves in profitable prey. We therefore also designed our experiments to evaluate the conditions under which aggregation will evolve in profitable prey.

Second, we note that while previous researchers addressed the possible advantages of aggregation in the evolution of aposematism, none of these tests presented aggregated and solitary configurations of unprofitable

prey simultaneously (Riipi et al. (2001) used combinations of aggregated and solitary prey in their detectability experiments, but these prey were all palatable). Perhaps a more appropriate approach would be to compare the survivorship of aggregated forms of unprofitable prey with that of solitary forms of unprofitable prey in the same system, as if a mutation for aggregation had recently arisen. We have therefore addressed this issue with our experiments, presenting solitary and aggregated forms of prey to predators simultaneously, thus allowing for comparison of the survivorship of prey in these two configurations, both between and within experiments.

## METHODS

The human predators (70 student volunteers) used in these experiments had no knowledge of the experimental aims (the majority were nonbiologists). No volunteer participated more than once, performing only a single replicate of one experiment. Their foraging environment consisted of a 14 × 14-cm white arena on a computer screen. All prey items were 5 × 5 mm, and labelled with either a cross or a black square. Prey items (and background items where appropriate) that were labelled with a cross were enclosed in a square border to aid the user in determining the extent of the prey. Distributed within the arena were background items (either crosses or squares, whichever was the symbol on the 'cryptic' form in a particular simulation). Also distributed in the arena were 16 'palatable' (profitable) prey, and 16 'unpalatable' (unprofitable) prey (see below). Prey were distributed on a randomized grid in the arena, with the constraint that no prey item or background item was allowed to overlap with another item.

Prey could be attacked by moving a cursor using a mouse, and clicking when the cursor was directly over the prey (an 'attack'). Human predators that attacked a profitable ('palatable') prey item received 1 point, those that attacked an unprofitable ('unpalatable') prey item lost 1 point. To indicate the profitability of an individual prey item (without requiring the predator to continually look at the score), attacks on profitable and unprofitable prey generated distinct sounds. Prey that were killed simply disappeared from the screen. Attacks on background items generated no response.

Each participant first took part in a single practise trial lasting 20 s, which was administered to familiarize participants with the test conditions and to minimize any effects of neophobia. Here, 16 cryptic and 16 conspicuous prey along with 16 cryptic and 16 conspicuous background items were distributed in the arena. All prey were profitable and solitary.

After the practise trial, each volunteer took part in a version of one of three experiments, which consisted of three consecutive trials. Each trial had 16 background items (experiment 1) or nine background items (experiments 2 and 3) (see below for justification), and all started with 16 profitable prey and 16 unprofitable prey distributed within the arena under the same experimental conditions. Each trial lasted for 20 s. After 20 s had

expired, a message box prompted the participant to proceed to the next trial by clicking on a button, until the third trial was complete. Participants were instructed to maximize their total points by trying to attack profitable prey while avoiding unprofitable prey. Because the participants were volunteers, they were all eager to take part in the study and we posted top scores on each experiment to enhance their motivation. Since a desire to maximize points, rather than hunger, was the motivation for our human predators, the three trials of each replicate were performed without an intervening time period.

Three experiments were conducted. The structure and rationale behind each of these experiments are summarized in Fig 1. Each version of each experiment was replicated seven times. For experiment 1 (the ‘initial origin’ experiments), test conditions were identical to those performed by Alatalo & Mappes (1996). Thus, in experiments 1a and 1b, solitary prey were distributed in the arena, with 16 profitable cryptic prey (similar to the background), eight unprofitable cryptic prey and eight unprofitable conspicuous prey (different from the background). Crosses were the background item in experiment 1a, whereas squares were the background item in experiment 1b. Experiments 1c and 1d were similar to experiments 1a and 1b, except that prey

were aggregated in four groups of four, with cryptic profitable, cryptic unprofitable and conspicuous unprofitable items grouped together. Crosses were the background item in experiment 1c; squares were the background item in experiment 1d.

In our further experiments (2a–d and 3a, b) 16 profitable and 16 unprofitable prey were again presented to predators in each of three trials. In these experiments, however, one of the prey types was presented such that half of the prey were in a solitary configuration (8 individuals) and half were in an aggregated configuration (two clusters of four). In experiments 2a–d, unprofitable prey were presented in these configurations; in experiment 3a–b, profitable prey were presented in this way. In experiment 2a–d, unprofitable prey conspicuousness (cross or square) and profitable prey configuration (solitary or aggregated) were systematically manipulated to study the interactions between these factors and unprofitable prey survivorship. In experiments 3a and 3b, the survivorship of solitary and aggregated profitable prey was compared under different configurations (solitary or aggregated) of unprofitable prey.

Background items were distributed in a solitary configuration throughout all these experiments. For experiments 2a and 2b, all of the profitable and half of the

Experiment	Background	Profitable		Unprofitable		Question
		(N = 8)	(N = 8)	(N = 8)	(N = 8)	
1a	☒	☒	☒	☒	■	Do we get similar results using humans rather than birds?
1b	■	■	■	■	☒	See above
1c	☒	☒☒ ☒☒	☒☒ ☒☒	☒☒ ☒☒	■■ ■■	See above
1d	■	■■ ■■	■■ ■■	■■ ■■	☒☒ ☒☒	See above
2a	☒	☒	☒	☒	☒☒ ☒☒	(1) Will aggregation evolve in cryptic unprofitable prey if it reliably indicates unprofitability?
2b	☒	☒	☒	■	■■ ■■	(2) Will aggregation evolve in conspicuous unprofitable prey if it is a reliable (yet superfluous) indicator of unprofitability?
2c	☒	☒☒ ☒☒	☒☒ ☒☒	☒	☒☒ ☒☒	(3) Will cryptic unprofitable prey evolve to be solitary if cryptic profitable prey are aggregated?
2d	☒	☒☒ ☒☒	☒☒ ☒☒	■	■■ ■■	(3) Will conspicuous unprofitable prey evolve to be solitary if cryptic profitable prey are aggregated?
3a	☒	☒	☒☒ ☒☒	■	■	(4) Will aggregation evolve in profitable prey when conspicuous unprofitable prey are solitary?
3b	☒	☒	☒☒ ☒☒	■■ ■■	■■ ■■	(4) Will aggregation evolve in profitable prey when conspicuous unprofitable prey are aggregated?

**Figure 1.** A summary of experimental conditions. A total of 16 individuals of each prey type (profitable and unprofitable) were presented at the beginning of each trial. Each prey type has been presented here in two columns (8 prey in each column per trial) to more easily portray the combinations of prey in each experiment. A total of 16 background items were distributed in experiment 1; nine background items were distributed in experiments 2 and 3. Questions 1–4 refer to similar questions posed in the Introduction.

unprofitable prey were distributed in a solitary, random configuration. For experiment 3a, all of the unprofitable and half of the profitable prey were distributed in the same way. This large number of solitary prey on the screen would sometimes cause the two aggregations of unprofitable prey to appear indistinct. Because we wanted aggregations of prey to appear distinctly as aggregations, the number of background items distributed on the screen was reduced from 16 (in experiment 1) to nine (in experiments 2 and 3). This ensured that prey items in an aggregated configuration were easily identified as aggregations.

In preparation for statistical analyses, we tested whether the survivorship of solitary prey was correlated with the survivorship of aggregated prey within each trial of each experiment (for unprofitable prey in experiment 2 and profitable prey in experiment 3). No significant correlations were found, so data on survivorship for within-experiment comparisons were analysed assuming independence.

## RESULTS

### Experiment 1: Great Tits versus Humans

Alatalo & Mappes (1996) reported the relative mortality of each prey type by calculating the proportion of killed prey items in each group (aposematic, unpalatable cryptic and palatable cryptic) divided by the randomly expected proportions initially available (0.25 for aposematic and unpalatable cryptic items, and 0.50 for profitable cryptic items), a 'mortality index'. In their calculations only the first six prey attacked in trial I and the first 12 prey attacked in each of trials II and III were included (see Alatalo & Mappes 1996, page 709). For ease of comparison we have begun by presenting the results of the 'initial origin' portion of our work (experiments 1a–d) in precisely this same manner, although we note that this 'mortality index', based on all prey rather than just unpalatable prey, may have interpretive limitations (Tullberg et al. 2000; see Alatalo & Mappes 2000 for a reanalysis). Our results with human predators foraging on computer-generated prey were surprisingly similar to those found with great tits (Fig. 2).

To examine how the survivorship of prey varied with experimental factors, we used a repeated measures ANOVA on arcsine square-root transformed proportions of each prey type that survived (here the denominator was simply the number of prey items of a given type initially present). Table 1 presents the within- and between-subject factors, main effects and significant interactions for all repeated measures ANOVA analyses conducted. The type of background (cross or square) had no significant effect (aposematic prey,  $P = 0.32$ ; cryptic unprofitable prey,  $P = 0.82$ ; cryptic profitable prey,  $P = 0.90$ ) in our experiments. The proportion of aposematic prey that survived increased significantly with increasing trial number, but the proportion of these prey that survived was not significantly affected by prey configuration. Neither trial number nor configuration had significant effects on the proportion of

unprofitable cryptic prey that survived. The proportion of profitable prey that survived decreased significantly with increasing trial number and was significantly higher when prey were distributed in the solitary configuration. As will be seen, this important result concerning profitable prey is reflected in our later experiments.

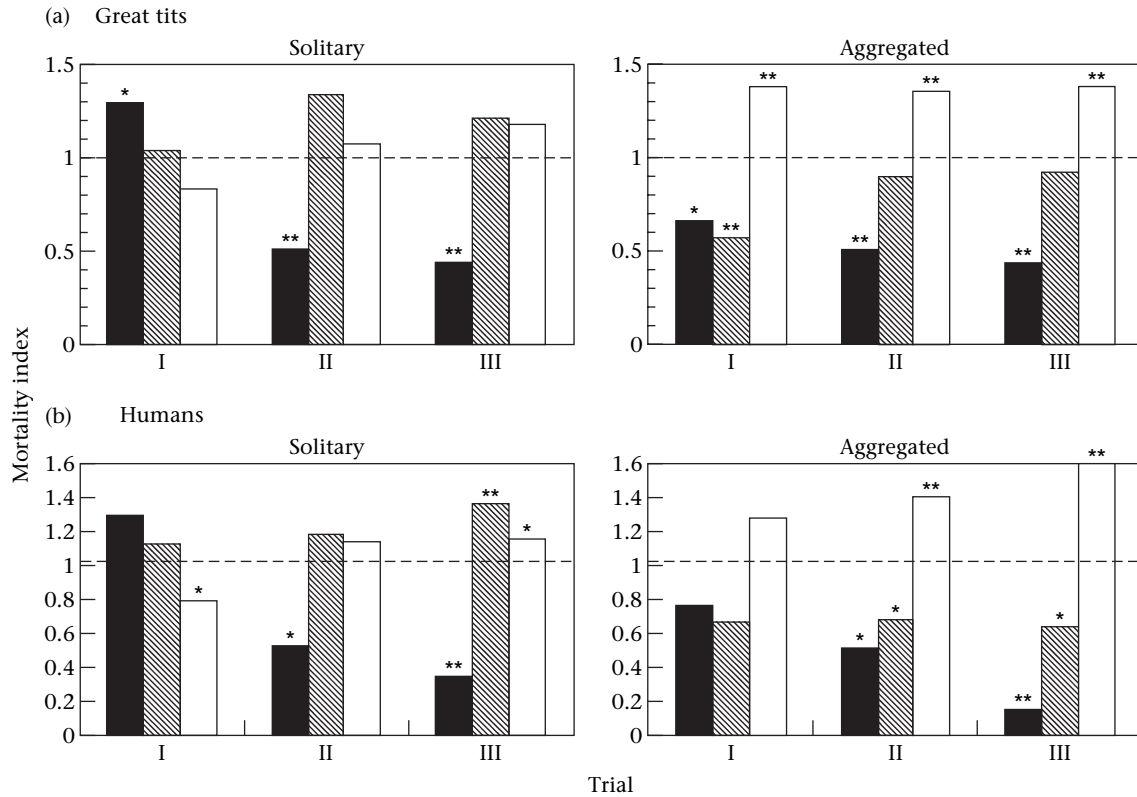
### Experiment 2: Solitary versus Aggregated Unprofitable Prey

Figure 3 shows the proportion of profitable and unprofitable forms of prey that survived per trial in experiments 2a–d. A repeated measures ANOVA revealed that the proportion of aggregated unprofitable prey that survived was significantly higher when these prey were conspicuous (and therefore looked different from profitable prey) rather than cryptic (experiments 2b and 2d versus experiments 2a and 2c) (Table 1). The proportion of these prey that survived also increased significantly with increasing trial number. There was no effect of the configuration of profitable prey on aggregated unprofitable prey survivorship, and no interactions were statistically significant.

The proportion of solitary unprofitable prey that survived was significantly higher when these prey were distinct in appearance (experiments 2c and 2d) rather than cryptic (experiments 2a and 2b), and increased significantly with increasing trial number (Table 1). In this case the proportion of solitary unprofitable prey that survived was also higher when profitable prey were aggregated rather than solitary, and there was a significant interaction effect between the aggregation of profitable prey and the conspicuousness of unprofitable prey. This interaction arose, at least in part, from the contrast between profitable and unprofitable prey: survivorship was higher for cryptic, solitary unprofitable prey when profitable prey were aggregated (experiment 2c versus experiment 2a). In summary, unprofitable prey in both configurations experienced increasing survivorship as trials advanced, and had higher survivorship when they looked different from profitable prey.

For profitable prey in these experiments (which were always cryptic), a repeated measures ANOVA (Table 1) revealed that the proportion that survived decreased significantly with trial number. Profitable prey had significantly lower survivorship when unprofitable prey were conspicuous (they were easily distinguished from unprofitable prey), and also had significantly lower survivorship when they were aggregated rather than solitary. There was also a significant interaction between the aggregation of profitable prey and the conspicuousness of unprofitable prey on the proportion of profitable prey that survived. In effect, profitable prey experienced lower survivorship when unprofitable prey were conspicuous, and much lower survivorship when profitable prey were aggregated, to the point of depletion of aggregated profitable prey (experiments 2c and 2d).

In experiment 2a, an independent-sample *t* test on arcsine square-root transformed proportions revealed that aggregated unprofitable prey had significantly higher



**Figure 2.** (a) Relative mortality index of aposematic (■), unpalatable cryptic (▨) and palatable cryptic (□) prey items when presented solitarily and aggregated in the 'initial origin' experiment of Alatalo & Mappes (1996) (an attacked prey item is counted as 'killed'). Mortalities are standardized by dividing the observed mortality by the randomly expected mortality; horizontal lines show the point at which observed matches expected mortality. Reprinted from Tullberg et al. (2000). (b) Relative mortality of aposematic (■), unprofitable cryptic (▨) and profitable cryptic (□) prey items in the computer version of the 'initial origin' experiment. Mortalities are calculated as in (a). Significant deviations from a randomly expected mortality of 1, as judged from separate *t* tests on arcsine transformed proportions, are denoted by asterisks (\* $P < 0.05$ ; \*\* $P < 0.01$ ).

survivorship than solitary unprofitable prey within trials II and III ( $t_{5,12} = 4.531$ ,  $P = 0.001$ ;  $t_{5,12} = 2.489$ ,  $P = 0.03$ , respectively). In this case all aggregated prey were reliably unprofitable. In experiments 2b–d, no significant differences between proportions of solitary unprofitable and aggregated unprofitable prey that survived were found in any of the three trials.

### Experiment 3: Solitary versus Aggregated Profitable Prey

Figure 4 shows the proportion of solitary and aggregated profitable prey that survived in experiment 3, when unprofitable prey were either conspicuous and solitary (experiment 3a) or conspicuous and aggregated (experiment 3b). To examine survivorship of each type of profitable prey (aggregated or solitary), we once again used a repeated measures ANOVA for arcsine square-root transformed proportions of prey that survived (Table 1). For solitary and aggregated profitable prey, survivorship did not change significantly with increasing trial number and was not affected by the configuration of unprofitable prey. No interactions were significant.

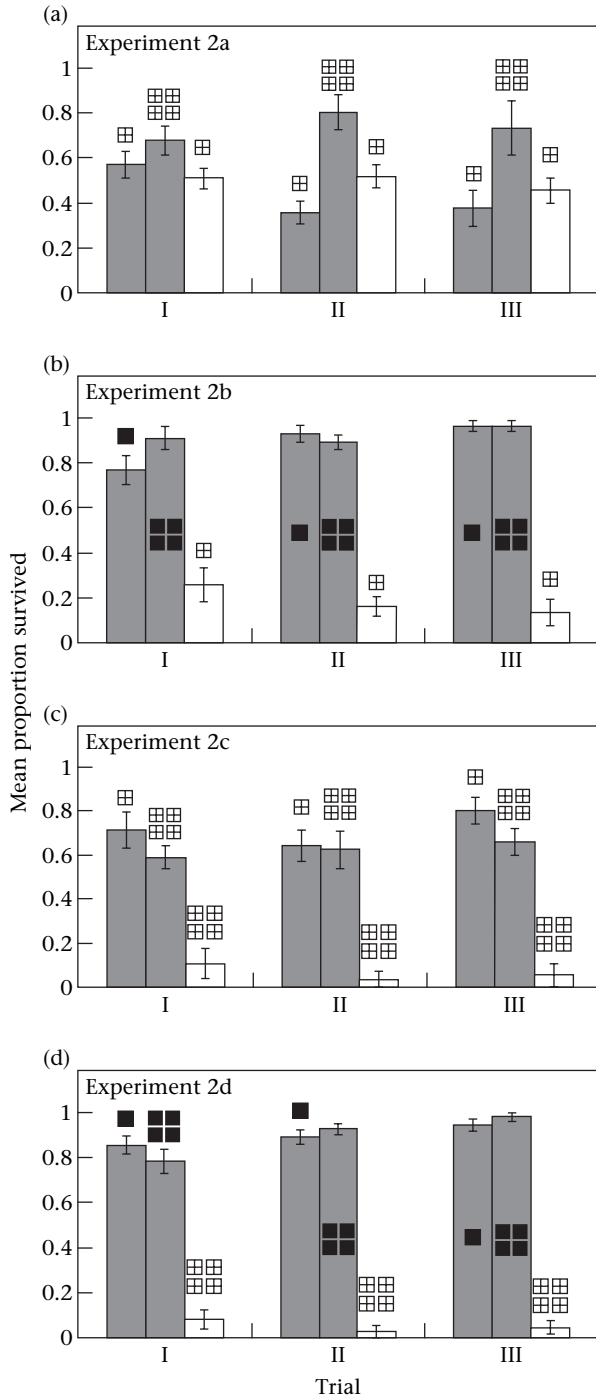
In experiment 3, 20 s was often adequate for a predator to deplete all aggregated profitable prey and then move on to solitary profitable prey, such that overall survivorship for solitary and aggregated profitable prey was approximately the same by the end of the trial. To demonstrate the depletion in these experiments, we show the path and attack order of predators (randomly selected from our data set) in trial III of experiments 3a and 3b (Fig. 5) (all replicates showed the same trend). Note that predators did not rapidly jump to different spatial locations over the screen, but (once aggregated profitable prey were removed) they moved around 'zapping' prey in a relatively smooth manner.

Depletion was not an issue in experiments 1 and 2, because profitable prey were not presented in different configurations within an experiment, and so switching from one profitable prey type to another could not occur. To address the issue of depletion of aggregated profitable prey in experiment 3, the survivorship of each prey type was analysed at 10 s into each trial. A repeated measures ANOVA for arcsine square-root transformed proportions of each prey item that survived found that the survivorship of aggregated profitable prey decreased significantly with increasing trial number, but was not affected by the configuration of unprofitable prey (always conspicuous in

**Table 1.** Results of repeated measures ANOVAs on arcsine transformed survivorship in experiments 1–3

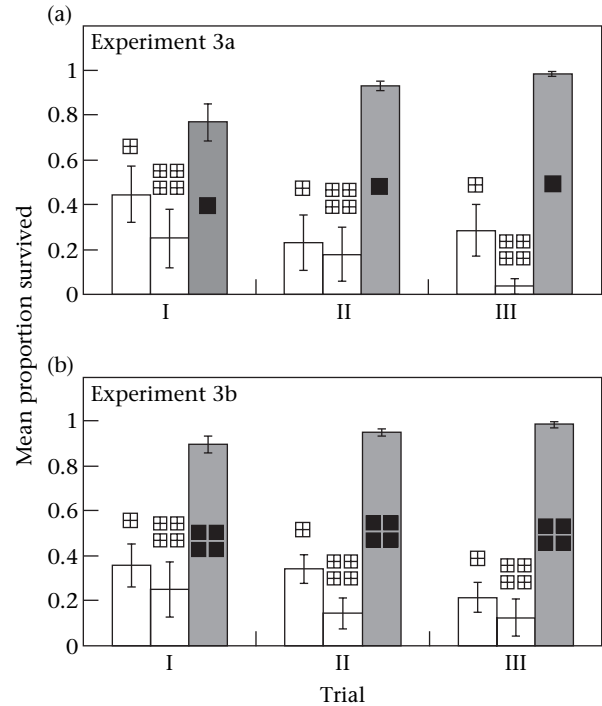
	Prey type	Main effects*				Significant interactions			
		Factors	<i>F</i>	df	<i>P</i>	Factors	<i>F</i>	df	<i>P</i>
Experiment 1	Aposematic	Trial (T)	7.88	2, 46	0.001				
		Background (B)	1.05	1, 23	0.32				
		Prey configuration (PC)	0.148	1, 23	0.70				
	Cryptic unprofitable	T	0.1	2, 46	0.91				
		B	0.06	1, 23	0.82				
		PC	0.05	1, 23	0.82				
	Cryptic profitable	T	4.38	2, 46	0.02				
		B	0.02	1, 23	0.90				
		PC	18.21	1, 23	<0.001				
Experiment 2	Solitary unprofitable	T	3.12	2, 46	0.05	PPC*CUP	10.10	1, 23	0.004
		Profitable prey configuration (PPC)	9.21	1, 23	0.006	T*CUP	5.63	2, 46	0.006
		Conspicuousness of unprofitable prey (CUP)	65.78	1, 23	<0.001	T*PPC*CUP	3.52	2, 46	0.04
	Aggregated unprofitable	T	5.12	2, 46	0.01				
		PPC	3.11	1, 23	0.09				
		CUP	29.90	1, 23	<0.001				
	Profitable	T	3.82	2, 46	0.03	PPC*CUP	8.11	1, 23	0.009
		PPC	42.30	1, 23	<0.001				
		CUP	7.90	1, 23	0.01				
Experiment 3 Full trial	Solitary profitable	T	2.96	2, 10	0.07				
		Unprofitable prey configuration (UPC)	0.29	1, 5	0.60				
	Aggregated profitable	T	3.14	2, 10	0.06				
		UPC	0.01	1, 5	0.91				
	Unprofitable	T	19.7	2, 10	<0.001				
		UPC	1.13	1, 5	0.31				
First 10 s	Solitary profitable	T	4.15	2, 10	0.03				
		UPC	0.001	1, 5	0.97				
	Aggregated profitable	T	4.19	2, 10	0.03				
		UPC	0.10	1, 5	0.76				
	Unprofitable	T	13.10	2, 10	<0.001				
		UPC	0.63	1, 5	0.44				

\*The within-subject factor in all experiments was trial (T). The between-subject factors in experiment 1 were background (B) and prey configuration (PC). The between-subject factors in experiment 2 were profitable prey configuration (PPC) and conspicuousness of unprofitable prey (CUP). The between-subject factor in experiment 3 was unprofitable prey configuration (UPC). Results for experiment 3 are presented for both the full trial and only the first 10 s of each trial.



**Figure 3.** Mean  $\pm$  SE proportions of prey that survived by trial in (a) experiment 2a, (b) experiment 2b, (c) experiment 2c and (d) experiment 2d. ■: unprofitable prey; □: profitable prey. Prey conspicuousness (inconspicuous: ☐; conspicuous: ■) relative to the background (☐) and prey configuration (solitary or aggregated) are represented by symbols above or within each bar.

these experiments). Solitary profitable prey survivorship also decreased significantly with increasing trial number and was not affected by the configuration of unprofitable prey. Independent-sample  $t$  tests revealed that aggregated profitable prey had significantly lower survivorship than



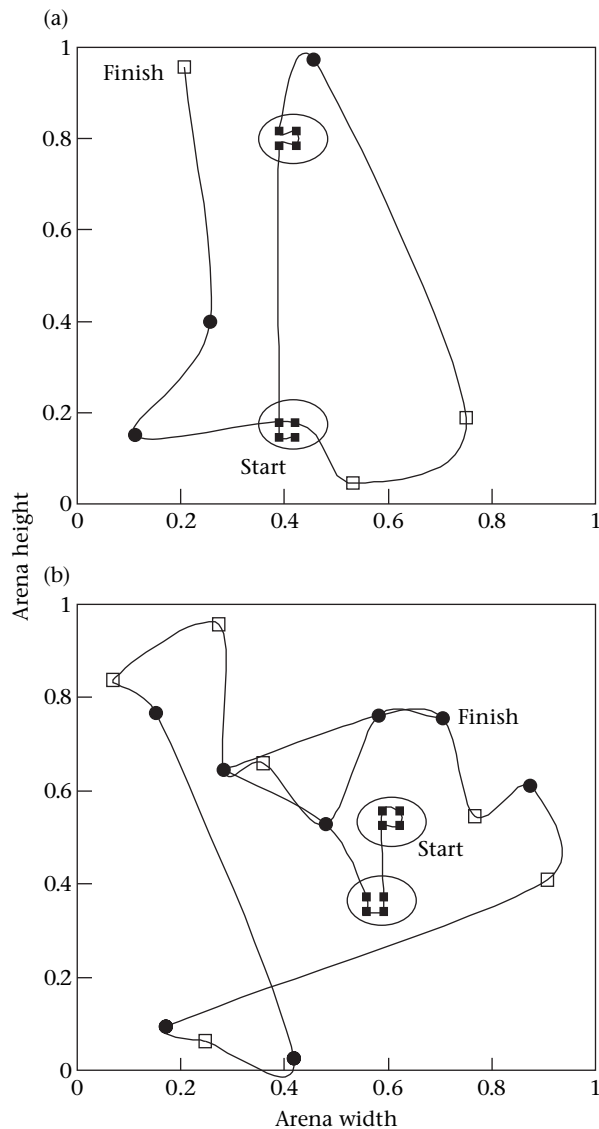
**Figure 4.** Mean  $\pm$  SE proportions of prey that survived by trial in (a) experiment 3a and (b) experiment 3b. In experiment 3a, unprofitable prey were conspicuous and solitary; in experiment 3b, unprofitable prey were conspicuous and aggregated. ■: unprofitable prey; □: profitable prey. Prey conspicuousness (inconspicuous: ☐; conspicuous: ■) relative to the background (☐) and prey configuration (solitary or aggregated) are represented by symbols above or on each bar.

solitary profitable prey within trial III of experiment 3a ( $t_{s,12} = 3.73$ ,  $P = 0.003$ ); a similar pattern with borderline significance was found within trial III of experiment 3b ( $t_{s,12} = 2.08$ ,  $P = 0.06$ ).

## DISCUSSION

Our application of the 'novel world' methodology using humans has revealed new insights about profitability, aggregation and predation. When we replicated the test conditions of Alatalo & Mappes (1996), humans demonstrated a striking similarity to previous data generated with bird predators. Two key reasons for this similarity are that both humans and birds are capable of responding to features that reliably distinguish profitable from unprofitable prey, and that both react in qualitatively similar ways to clusters of prey. In particular, while both great tits and humans would leave an aggregation of unprofitable prey after attacking the first item in the aggregation, they would systematically deplete aggregations of profitable prey.

Our new experiments do not unequivocally support the contention that aggregation is a necessary step in the evolution of aposematism. Aggregated, cryptic, unprofitable prey did eventually experience a significantly higher survivorship than solitary, cryptic, unprofitable prey, but only when this aggregation allowed them to be



**Figure 5.** Plot of the path taken by predators in attacking prey in (a) experiment 3a, trial III and (b) experiment 3b, trial III (arena dimensions were  $14 \times 14$  cm; dimensions are scaled to 1). These replicates were chosen at random from the seven replicates of each experiment (all of the replicates were similar). ■: aggregated profitable prey that were attacked; □: solitary profitable prey that were attacked; ●: background items that were attacked. Aggregations of profitable prey are indicated with ovals; the start and finish points of the predator path are labelled.

distinguished from profitable prey (experiment 2a). Our results suggest that if prey palatability can be assessed on the basis of aggregation alone, then there may be less of a selective advantage in evolving a separate aposematic signal. Indeed, to take the converse, aggregated and solitary unprofitable prey did not have any marked differences in survivorship when unprofitable prey could be distinguished on the basis of their conspicuousness (experiment 2b). The tendency, albeit nonsignificant, for unprofitable cryptic prey to survive better when they were solitary only when profitable prey were aggregated (experiment 2c), and the significant effect of configuration of

profitable prey on attack rate of solitary unprofitable prey (ANOVA results, see Table 1), once again support the contention that unprofitable prey may evolve characteristics that help predators distinguish them from profitable prey (Fisher 1930; Srygley & Chai 1990; Sherratt 2002; Sherratt & Beatty 2003; Sherratt et al. 2004; Srygley 2004).

Some researchers have suggested a potential benefit of gregariousness to aposematic prey (Gagliardo & Guilford 1993; Gamberale & Tullberg 1996, 1998; Riipi et al. 2001; Sherratt 2002) in that aposematic prey in aggregations might present a heightened signal, and thereby be more repellent to potential predators. After directly comparing the survivorship of solitary and aggregated aposematic prey (experiments 2b and 2d) within all three trials, we could find no evidence in our particular study that humans were more effectively deterred by aggregated compared to solitary conspicuous signals. In our case, when the profitability of prey was reliably signalled through prey conspicuousness (profitable prey were never conspicuous), then both forms were left relatively untouched from an early stage. One reason for this discrepancy may be that our human predators learned quickly to avoid reliably unprofitable prey types, whatever they looked like. It is also possible that our initial instruction trial reduced any potential heightened response to conspicuous signals. Finally, we note that our conspicuous prey were black squares, and did not show a more natural warning signal.

While most researchers have focused on what traits are advantageous in unprofitable prey, here we have also considered what traits are advantageous in profitable prey. When solitary and aggregated profitable prey were presented to predators simultaneously, aggregated profitable prey were attacked more than solitary profitable prey, regardless of whether unprofitable prey were solitary or aggregated (experiment 3, 10-s trials). Further support for this pattern can be seen by comparing the fate of aggregated and solitary profitable prey within experiment 2. In this experiment, aggregated profitable prey were attacked at a much higher rate than solitary prey, with aggregated profitable prey consistently being depleted (Fig. 3). The process by which this occurred is well demonstrated in Fig. 5, which shows the foraging paths taken by our human predators in experiment 3. Predators rapidly moved to aggregated profitable prey first in these trials, and consequently consumed all members of an aggregation before moving on. In experiment 3b, unprofitable prey were also aggregated, but in contrast to profitable prey, predators attacking an unprofitable prey item in an aggregation would quickly leave the aggregation.

With respect to our initial questions (1–4) the overwhelming pattern that emerges from all of these experiments is a highly intuitive one. Unprofitable prey had higher survivorship when they had traits that helped distinguish them from profitable prey, a case that is supported by both between- and within-experiment comparisons of our results. When unprofitable prey could be distinguished from profitable prey on the basis of their appearance (e.g. conspicuousness), then there was no significant advantage to them adopting one configuration over another. Leaving aside the possibility of perfect mimicry of unprofitable aggregated prey, profitable prey

tend to survive better on a per capita basis when solitary rather than in aggregated form for the simple reason that aggregations of profitable prey represent rich sources of prey that are preferentially exploited. In nature, without the satiating effect of distastefulness, small, edible prey are at a disadvantage in that their aggregations would need to be infinitely large for individual survivorship to be enhanced by predator satiation (Sillén-Tullberg & Leimar 1988). Thus aggregation is severely limited as a means of predation avoidance for small profitable prey.

Phylogenetic studies (Sillén-Tullberg 1988; Tullberg & Hunter 1996; Nilsson & Forsman 2003) have revealed that the majority of repellent defences and warning colours have evolved in species that continue to be solitary. For instance, in their sample of 578 species of moth, Nilsson & Forsman (2003) found that only 34% of species with conspicuous larvae show an aggregated lifestyle, whereas less than 2% of species with nonconspicuous larvae are aggregated. Tullberg & Hunter (1996) examined a sample of 800 tree-living macrolepidopteran larvae and argued that their data were consistent with the proposal that the evolution of repellent defences and warning coloration preceded the evolution of gregariousness. Our experiments do not reveal anything directly about the order in which traits such as conspicuousness and gregariousness evolved, but they do show that nonmimetic profitable prey tend to be vulnerable when in aggregations, and that unprofitable prey can sometimes, but not always, gain from being clustered.

Of course, there are more general benefits of gregariousness, regardless of prey defence, such as thermal enhancement, increased developmental rate and protection from desiccation (Clark & Faeth 1997, 1998; Klok & Chown 1999; see Krause & Ruxton 2002 for a review). Because there are benefits to gregariousness not related to prey defence, could it be that unprofitable prey tend to be aggregated because they can avail themselves of these benefits, while profitable prey simply cannot? This could explain why some, but not all, aposematic species are aggregated, and why so few profitable species are found in aggregations (Nilsson & Forsman 2003). In other words, the observed association between gregariousness and aposematism might not be a function of prey defence in itself, but a case where selection against aggregation has been relaxed in unprofitable prey.

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### References

- Alatalo, R. V. & Mappes, J. 1996. Tracking the evolution of warning signals. *Nature*, **382**, 708–710.
- Alatalo, R. V. & Mappes, J. 2000. Initial evolution of warning coloration: comments on the novel world method. *Animal Behaviour*, **60**, F1–F2: <http://www.elsevier.com/locate/anbehav>.
- Bertram, B. C. R. 1978. Living in groups: predators and prey. In: *Behavioural Ecology: an Evolutionary Approach* (Ed. by J. R. Krebs & N. B. Davies), pp. 64–96. Sunderland, Massachusetts: Sinauer.
- Clark, B. R. & Faeth, S. H. 1997. The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, **22**, 408–415.
- Clark, B. R. & Faeth, S. H. 1998. The evolution of egg clustering in butterflies: a test of the egg desiccation hypothesis. *Evolutionary Ecology*, **12**, 543–552.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press.
- Gagliardo, A. & Guilford, T. 1993. Why do warning-colored prey live gregariously? *Proceedings of the Royal Society of London, Series B*, **251**, 69–74.
- Gamberale, G. & Tullberg, B. S. 1996. Evidence for a more effective signal in aggregated aposematic prey. *Animal Behaviour*, **52**, 597–601.
- Gamberale, G. & Tullberg, B. S. 1998. Aposematism and gregariousness: the combined effect of group size and coloration on signal repellence. *Proceedings of the Royal Society of London, Series B*, **265**, 889–894.
- Gittleman, J. L. & Harvey, P. H. 1980. Why are distasteful prey not cryptic? *Nature*, **286**, 149–150.
- Glanville, P. W. & Allen, J. A. 1997. Protective polymorphism in populations of computer-simulated moth-like prey. *Oikos*, **80**, 565–571.
- Hamilton, W. D. 1963. Evolution of altruistic behavior. *American Naturalist*, **97**, 354–356.
- Hamilton, W. D. 1996. *The Narrow Roads of Gene Land*. Oxford: W. H. Freeman.
- Klok, C. J. & Chown, S. L. 1999. Assessing the benefits of aggregation: thermal biology and water relations of anomalous emperor moth caterpillars. *Functional Ecology*, **13**, 417–427.
- Krause, J. & Ruxton, G. D. 2002. *Living In Groups*. Oxford: Oxford University Press.
- Nilsson, M. & Forsman, A. 2003. Evolution of conspicuous colouration, body size and gregariousness: a comparative analysis of lepidopteran larvae. *Evolutionary Ecology*, **17**, 51–66.
- Poulton, E. B. 1890. *The Colours of Animals: Their Meaning and Use Especially Considered in the Case of Insects*. London: Keegan Paul, Trench, Trübner.
- Riipi, M., Alatalo, R. V., Lindström, L. & Mappes, J. 2001. Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature*, **413**, 512–514.
- Sherratt, T. N. 2002. The coevolution of warning signals. *Proceedings of the Royal Society of London, Series B*, **269**, 741–746.
- Sherratt, T. N. & Beatty, C. D. 2003. The evolution of warning signals as reliable indicators of prey defense. *American Naturalist*, **162**, 377–389.
- Sherratt, T. N., Rashed, A. & Beatty, C. D. 2004. The evolution of locomotory behaviour in profitable and unprofitable simulated prey. *Oecologia*, **138**, 143–150.
- Sillén-Tullberg, B. S. 1988. Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution*, **42**, 293–305.
- Sillén-Tullberg, B. S. & Leimar, O. 1988. The evolution of gregariousness in distasteful insects as a defense against predators. *American Naturalist*, **132**, 723–734.

- Srygley, R. B.** 2004. The aerodynamic costs of warning signals in palatable mimetic butterflies and their distasteful models. *Proceedings of the Royal Society of London, Series B*, **271**, 589–594.
- Srygley, R. B. & Chai, P.** 1990. Predation and the elevation of thoracic temperature in brightly colored neotropical butterflies. *American Naturalist*, **135**, 766–787.
- Tucker, G. M. & Allen, J. A.** 1993. The Behavioral basis of apostatic selection by humans searching for computer-generated cryptic prey. *Animal Behaviour*, **46**, 713–719.
- 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. *Biological Journal of the Linnean Society*, **57**, 253–276.
- Tullberg, B. S., Leimar, O. & Gamberale-Stille, G.** 2000. Did aggregation favour the initial evolution of warning coloration? A novel world revisited. *Animal Behaviour*, **59**, 281–287.
- Wallace, A. R.** 1867. Untitled note. *Proceedings of the Entomological Society of London, March 4th*, 1xxx–1xxxi.