

Tracking the evolution of warning signals

Rauno V. Alatalo & Johanna Mappes

Department of Biological and Environmental Science, Konnevesi Research Station, University of Jyväskylä, PO Box 35, FIN-40351 Jyväskylä, Finland

EVOLUTIONARY studies are hampered by a lack of experimental ways in which to test past events such as the origination of aposematism¹⁻⁷, whereby unpalatable or poisonous prey signal their unprofitability, often by being warningly coloured. Inexperienced predators do learn to avoid unpalatable prey as a result of such signals⁸⁻¹⁰, but in addition there may be an inherited cautiousness about attacking when common or conspicuous warning signals are evident¹¹⁻¹⁶. As current predators are not naive in the evolutionary sense, it is still not resolved^{3-7,17,18} whether aposematism originated only in aggregations of prey^{19,20} or among solitary prey as well²¹⁻²³. Here we explore this controversy in evolutionarily naive predators by creating a novel world with warning signals not found in the environment. Initially, the aggregation of prey favoured the warning signals supporting Fisher's view²⁴ of kin aggregations as the evolutionary starting point of aposematism. However, once predators had experienced warning signals, pre-existing avoidance seemed to facilitate evolution of Müllerian mimicry complexes²⁵ with similar types of signals even among solitary prey.

On the basis of the fact that hand-reared predators with no experience of aposematic prey are not naive in the evolutionary sense, we created a world in which there were two types of symbols (crosses and filled squares) that were switched to form the background or the warning signal. As the symbols were not present during the evolutionary history of the predators, we assume that the experiments illustrate the initial conditions experienced by the first aposematic prey individuals. We started from the situation in which an unpalatable prey evolves a warning signal, then continued to test conditions for the evolution of similar signals in other unpalatable prey items (Müllerian mimicry)²⁵. We used great tits (*Parus major*) caught in the wild as predators. To enhance the image of a novel world, all prey items were artificial. As initial prey we used items made of a piece of rye straw (a hollow stem of dead cultivated rye) filled with animal fat. White paper 'wings' at each end of the straw piece had either a cryptic signal similar to the background or a warning signal different from the

background. To make the items unpalatable we added chloroquine to the fat. As secondary prey, we used pieces of almond with the symbol glued on the slice.

In the 'initial origin' experiment, the prey were either dispersed as single items or aggregated with four similar items clumped close together (Fig. 1). As the idea was to determine how the warning signal initially enhances the survival probability of prey that have just acquired unpalatability^{19,20}, we had three types of randomly distributed prey: palatable controls with the cryptic symbol (16 items), unpalatable prey with the cryptic symbol (8 items), and unpalatable prey with the symbol differing from that of the background as the aposematic prey (8 items). Each individual tit had been trained to eat straw items with the cryptic symbol, and they were allowed to search for one hour in the test room. The procedure was repeated on three consecutive days (trials I-III). Conspicuousness or the type of signal itself did not influence the innate preferences between the two types of signals. Another set of eight tits were presented with two prey items (only 2 cm apart) and the same choice situation was repeated four times. The birds did not prefer any of the symbols (first choices for squares, 15, and crosses, 17; binomial test, $P = 0.86$) or the symbol conspicuous-

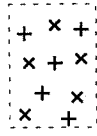






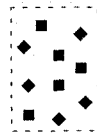






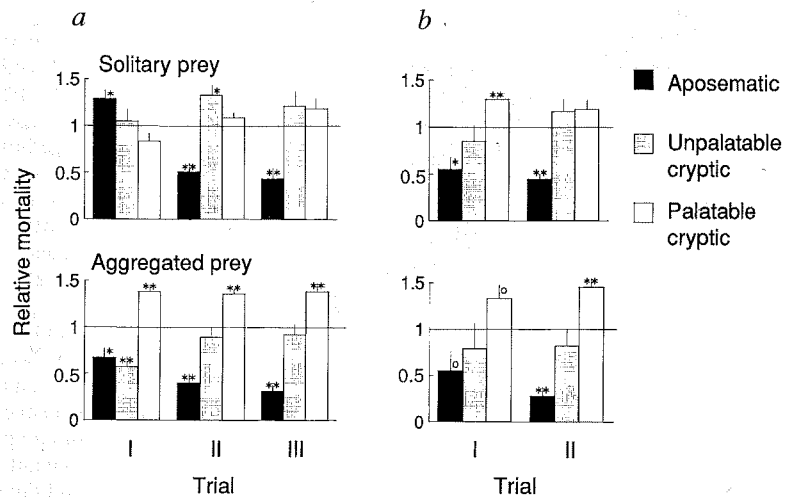
		Type of dispersion	
		Solitary prey	Aggregated prey
Background		 palatable 16x1	 palatable 4x4
		 unpalatable 8x1	 unpalatable 2x4
		 aposematic 8x1	 aposematic 2x4
		 palatable 16x1	 palatable 4x4
		 unpalatable 8x1	 unpalatable 2x4
		 aposematic 8x1	 aposematic 2x4

FIG. 1 The experimental set-up to test the origin of warning signals among straw prey. Each individual great tit was randomly assigned to one of the four treatments shown. The background was either a cross or a square and the prey were presented either solitary or in aggregation. In each case, there were 16 palatable cryptic prey, 8 unpalatable cryptic prey and 8 unpalatable signalling prey (aposematic). Palatable items consisted of animal fat in a 6-mm rye straw with paper wings. Unpalatable items had a 12% concentration of chloroquine. Prey items were randomly placed on a 2 × 2 m floor of an aviary, where there were 6 × 6 = 36 pieces of white A4 paper (21 × 30 cm) with symbols as the background. To train the tits to use their cryptic items, they were offered two palatable pieces of straw filled with fat on two consecutive days. Before the trials, the tits were not allowed to feed for two hours, and each trial lasted one hour. We used only the 12 first items in the analyses, but in the first trials only the 6 first items were included to obtain the initial predation risks before birds could learn the signals. In the second experiment, almond slices were reduced to ~ 6 × 6 mm, with symbols glued to each slice with non-toxic glue. Unpalatable slices were dipped in a 40% solution of chloroquine.

FIG. 2 Relative mortality for aposematic, unpalatable cryptic and palatable cryptic prey items when presented solitary or aggregated. The proportions of killed items in each group were divided by the randomly expected proportions, which were 0.25 for aposematic and unpalatable cryptic items, and 0.50 for palatable cryptic items. In this way, the average mortality over the three types of items is standardized to unity. In the first stage (a) with artificial straw prey, the experiment was repeated three times with the same birds, and in the second stage (b), the same predator individuals each encountered a similar set-up of slices of almond with similar signals in two consecutive trials. Standard errors are shown; asterisks indicate two-tailed significance (circle symbol, $P < 0.10$; * $P < 0.05$; ** $P < 0.01$) of a difference from the average mortality of all items, scaled to unity as indicated by the horizontal lines. We used repeated measures ANOVA for arcsin-square-root transformed proportions of each type of item to be 'killed'. The type of background (square or cross) had no effect in either setup for any of the three prey types ($P > 0.50$), so for simplicity we have combined the presentation of data. In the 'initial origin' experiment (a), for aposematic items both the type of prey dispersion ($F_{(1,23)} = 8.47, P < 0.01$) and the trial number ($F_{(2,46)} = 12.10, P < 0.01$) indicated significant effects. Likewise, type of prey dispersion ($F_{(1,23)} = 15.86, P < 0.010$) and trial number ($F_{(2,46)} = 5.32, P < 0.01$) had a significant effect on the proportion of unpalatable cryptic items being killed. For the cryptic palatable items, the type of dispersion was significant ($F_{(1,23)} = 24.38, P < 0.001$) but trial



number was not ($F_{(2,46)} = 2.39, P < 0.10$). In the 'secondary origin' experiment (b) the proportion of aposematic items handled was not significantly dependent on the type of prey dispersion ($F_{(1,17)} = 1.85, P = 0.19$, repeated measures ANOVA) or the trial number ($F_{(1,17)} = 0.37, P = 0.58$).

ness (similar to background, 16; different from background, 16; binomial test, $P = 1.00$).

In the 'initial origin' experiment, if the prey were aggregated, tits left the unpalatable groups after tasting the first prey items (Fig. 2a, trial I). Thus, the selective advantage of aggregation appeared initially for both the aposematic prey and the unpalatable cryptic prey. In later trials (II–III), whereas unpalatable cryptic items retained their advantage over palatable cryptics, aposematic items were used much less as a consequence of birds learning to avoid the signalling prey. If the prey were solitary, there was initially a disadvantage for warning signals (trial I), but selective advantage appeared in later trials after predators had previous experience with similar phenotypes. Altogether, this suggests that initially aggregation would have been beneficial for the aposematic prey, seemingly supporting Fisher's view of kin selection. Indeed, unpalatability alone would have selected for gregariousness which subsequently facilitates the evolution of warning signals. Although our experiment assured that the signals were novel, the predators could have experienced aggregated unpalatable prey in the wild, and aggregation alone might act as a cue of unpalatability^{2,4,26}. However, we tested aggregated unpalatable prey together with aggregated palatable prey, so tits never had a choice between aggregated and solitary items. Furthermore, among the first attacks only, the items with warning signals were equally often attacked first in both types of prey dispersions (in 9 out of 14 cases, an aposematic item was attacked before any of the unpalatable cryptic items among solitary prey, and in 10 out of 14 cases among aggregated prey).

In the second series of experiments, the same birds were feeding on similarly arranged artificial-prey items of sliced almonds. Thus, this prey had a totally different appearance, but the cryptic and aposematic symbols were exactly the same as in the initial prey. Within the first trial, the risk of being 'killed' was highly reduced among the dispersed prey items (Fig. 2b). Predators had avoided the signalling, unpalatable items in the first encounters, suggesting that, in Müllerian mimicry, aggregation of the prey is not essential. Thus, the controversy^{2-7,14-22,26,27} over the role of prey aggregation may well originate from different conditions in previous experiments. If the situation was unusual, then the necessity of aggregation may have been important, but if the experiments used traditional signals, the benefit could have appeared even among the solitary prey items. In our experiment, the signal was exactly

the same but the prey items were very different in appearance. Thus, Müllerian mimicry may also operate effectively for imperfect mimics, which indeed may have been the scenario in which many of the presently occurring aposematic forms evolved.

Our experiments suggest a possible route for the initial evolution of aposematism in the world with evolutionarily naive predators. Unpalatability alone selected for gregariousness, as predators would just leave the aggregations after tasting the first items. Once the prey were aggregated, selection would have instantly favoured the appearance of a warning signal to allow the predators to learn to associate the signal with unpalatability. The assumption that aggregation and kin grouping would have been closely associated makes sense, because an aggregation may arise from the habit of a single female to lay her clutch in one place²⁸. However, it is not obvious that we need to invoke kin selection to explain the evolution of warning signals^{4,18}. It is the aggregation itself that helps the already unpalatable aposematic prey types. Finally, once predator populations have experienced the world with aposematic prey, aggregations may not be necessary for successful evolution of common warning signals in a new species. □

Received 25 April; accepted 28 June 1996.

- Poulton, E. B. *The Colours of Animals: Their Meaning and Use* (Kegan Paul, Trench, Trubner, London, 1890).
- Edmunds, M. *Defence in Animals: A Survey of Anti-predator Defences* (Longman, Harlow, Essex, 1974).
- Guilford, T. *Am. Nat.* **131**, S7–S21 (1988).
- Guilford, T. in *Insect Defence: Adaptive Mechanisms and Strategies of Prey and Predators* (eds Evans, D. L. & Schmidt, J. O.) 23–61 (State Univ. of New York Press, NY, 1990).
- Mallet, J. & Singer, M. C. *Biol. J. Linn. Soc.* **32**, 337–350 (1987).
- Endler, J. A. *Phil. Trans. R. Soc. Lond. B* **319**, 505–523 (1988).
- Endler, J. A. in *Behavioural Ecology: An Evolutionary Approach* 3rd edn (eds Krebs, J. R. & Davies, N. B.) 169–196 (Blackwell, Oxford, 1991).
- Gittleman, J. L. & Harvey, P. H. *Nature* **286**, 149–150 (1980).
- Roper, T. J. & Wistow, R. Q. *J. exp. Psychol.* **38**, 141–149 (1986).
- Roper, T. J. & Redston, S. *Anim. Behav.* **35**, 739–747 (1987).
- Rubinoff, I. & Kropach, C. *Nature* **228**, 1288–1290 (1970).
- Smith, S. M. *Science* **187**, 759–760 (1975).
- Smith, S. M. *Nature* **265**, 535–536 (1977).
- Sillén-Tullberg, B. *Oecologia* **67**, 411–415 (1985).
- Wiklund, C. & Järvi, T. *Evolution* **36**, 998–1002 (1982).
- Schuler, W. & Hesse, E. *Behav. Ecol. Sociobiol.* **16**, 249–255 (1985).
- Malcolm, I. S. B. *Trends Ecol. Evol.* **5**, 57–62 (1990).
- Guilford, T. *Oikos* **45**, 31–36 (1985).
- Harvey, P. H. & Paxton, R. J. *Oikos* **37**, 391–393 (1981).
- Harvey, P. H., Bull, J. J., Pemberton, M. & Paxton, R. J. *Am. Nat.* **119**, 710–719 (1982).
- Järvi, T., Sillén-Tullberg, B. & Wiklund, C. *Oikos* **36**, 267–272 (1981).
- Sillén-Tullberg, B. & Bryant, E. H. *Evolution* **37**, 993–1000 (1983).

- 23. Sillén-Tullberg, B. *Evolution* **42**, 293–305 (1988).
- 24. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, Oxford, 1930).
- 25. Müller, F. *Proc. ent. Soc. Lond.* **1879**, 20–29 (1879).
- 26. Gagliardo, A. & Guilford, T. *Proc. R. Soc. Lond. B* **251**, 69–74 (1993).
- 27. Sillén-Tullberg, B. *Anim. Behav.* **40**, 856–860 (1990).
- 28. Stamp, N. E. *Am. Nat.* **115**, 367–380 (1980).

ACKNOWLEDGEMENTS. We thank M. Elgar, T. Guilford, T. Horne, L. Lindström, J. Mallet, A. P. Møller, R. Paul, A. Rivero, B. Sinervo, B. Tullberg and J. Tuomi for many valuable comments, and the staff at the Konnevesi Research Station and M. Einbork for technical support. This study was supported by the Adacemy of Finland.

CORRESPONDENCE and requests for materials should be addressed to R.V.A. (e-mail: alatalo@jytk.jyu.fi).

Tracking the evolution of learning signals

R. V. ALATALO & M. SILLÉN-TULLBERG

Learning signals are used by many animals to indicate the location of food or other resources. The evolution of learning signals is often assumed to be driven by the need to attract conspecifics to food resources. However, we show that learning signals can also evolve as a result of the need to attract predators. We present evidence from a field study of the evolution of learning signals in the common cuckoo (*Cuculus fulvus*) that supports this hypothesis. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy.

The evolution of learning signals is often assumed to be driven by the need to attract conspecifics to food resources. However, we show that learning signals can also evolve as a result of the need to attract predators. We present evidence from a field study of the evolution of learning signals in the common cuckoo (*Cuculus fulvus*) that supports this hypothesis. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy.

The evolution of learning signals is often assumed to be driven by the need to attract conspecifics to food resources. However, we show that learning signals can also evolve as a result of the need to attract predators. We present evidence from a field study of the evolution of learning signals in the common cuckoo (*Cuculus fulvus*) that supports this hypothesis. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy.

Evolution of learning signals

The evolution of learning signals is often assumed to be driven by the need to attract conspecifics to food resources. However, we show that learning signals can also evolve as a result of the need to attract predators. We present evidence from a field study of the evolution of learning signals in the common cuckoo (*Cuculus fulvus*) that supports this hypothesis. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy.

The evolution of learning signals is often assumed to be driven by the need to attract conspecifics to food resources. However, we show that learning signals can also evolve as a result of the need to attract predators. We present evidence from a field study of the evolution of learning signals in the common cuckoo (*Cuculus fulvus*) that supports this hypothesis. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy. The evolution of learning signals in the common cuckoo is driven by the need to attract predators, rather than by the need to attract conspecifics. This is because the evolution of learning signals in the common cuckoo is associated with the evolution of a specific anti-predator strategy.