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Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks

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Colours are common stimuli in signalling systems. Requirements to function well as a signal sometimes conflict between different signallers, and the same colour stimulus is used to convey completely different messages to the same receiver. Fruits and aposematic insects both use red coloration as a signal, in the former case to signal profitability and in the latter case as a warning signal. In two experiments, we investigated whether the domestic chick, an omnivorous predator, differed in its unconditioned preference or avoidance of red and green stimuli depending on whether or not the stimulus was an insect. The experiments were designed as preference tests between red and green painted prey. The prey were live insects and artificial fruits (experiment 1), and, to investigate the effect of movement, live and dead insects (experiment 2). The chicks did not show any difference in pecking preference between red and green when fruit-like stimuli were used, but when the prey were insects, green prey were strongly preferred to red prey, and prey movement did not affect this bias. Thus, young chicks may recognize prey as insects and then discriminate between different prey colorations, or one type of food may elicit an unlearned colour preference–avoidance response that is absent with another type of food.

Keywords: aposematism; colour signals; frugivory; foraging; innate bias; receiver psychology

1. INTRODUCTION

Colour signals are often used in communication systems in nature. They are, for example, used to confer messages between individuals of the same species, as in mate choice or parent–offspring communication, as well as between species, for example by plants to attract pollinators, or by aposematic prey to signal unpalatability to potential predators. Independently of context, all signals need to be designed to meet similar requirements to function well for their purpose. A signal needs to be easy to detect and to be recognised and learned by the receiver of the signal. Thus, the psychology and physiology of the receiver have an important effect on what signal design features are beneficial (Guilford & Dawkins 1991). Moreover, selection pressures may also act on the receivers, as they may benefit from effective responses to the signals, suggesting signal–receiver coevolution. Studies on birds' unlearned responses to colours and patterns used in warning coloration by poisonous snakes and noxious insects strongly suggest that this may be the case (reviewed in Schuler & Roper 1992).

However, the same colour stimulus is often used in different signalling systems, and, when the intended receiver is the same, it may happen that the same colour is used to confer conflicting messages from different senders (Schuler & Roper 1992). For instance, red coloration is often used to signal avoidance in the case of warning coloration (Cott 1940; Edmunds 1974; Guilford 1990) and, at the same time, it is a common fruit colour that works to attract frugivorous birds and mammals for seed dispersal (Ridley 1930; Willson 1990; Puckey *et al.* 1996). It could be argued that many ripe fruits also have a significant ultraviolet component in their signals, and thereby they may differ from insects. However, although this is common for blue and black fruits, red fruits rarely reflect in the ultraviolet (Willson & Whelan 1989). Thus, individual predators that feed on both fruits and arthropods would not benefit from avoiding red food items altogether, but should show discrimination between food types in their reactions to colour stimuli.

Experiments investigating unlearned feeding preferences or aversions in birds towards red coloration are somewhat inconsistent (Schuler & Roper 1992; Roper & Marples 1997*a*; Gamberale-Stille & Tullberg 1999). There are several possible reasons for this inconsistency. For instance, the actual spectral compositions and the sizes of the stimuli may vary between experiments, and these characteristics may be important in the bird's reactions to visual stimuli (Osorio *et al.* 2001). Another reason for the inconsistency could be that birds differ in their pecking preferences for different colours depending on whether or not the item is an insect. For instance, when presented with illuminated spherical discs or small beads, chicks prefer red or blue (Fisher *et al.* 1975; Rogers 1995 and references therein). On the other hand, when presented with insects, chicks seem to be relatively averse to red (Roper & Cook 1989; Roper 1990). Roper & Marples' (1997*a*) experiments showed that the mode of presentation may affect chick colour preferences. When presented in a pile on the floor, red crumbs were preferred to green and black, but when presented in a Petri dish, red crumbs elicited the lowest response. Additionally, other prey attributes, such as a warning odour, may affect colour preferences in chicks (Marples & Roper 1996; Rowe & Guilford 1996). For instance, in naive chicks, the presence of pyrazine odour, often used in insect defence systems, may elicit a relative avoidance of crumbs with a common warning colour, such as red and yellow, compared with green (Rowe & Guilford 1996, 1999), which is not as common in warning displays (Edmunds 1974). Thus, it seems that birds' unlearned reactions to a colour stimulus

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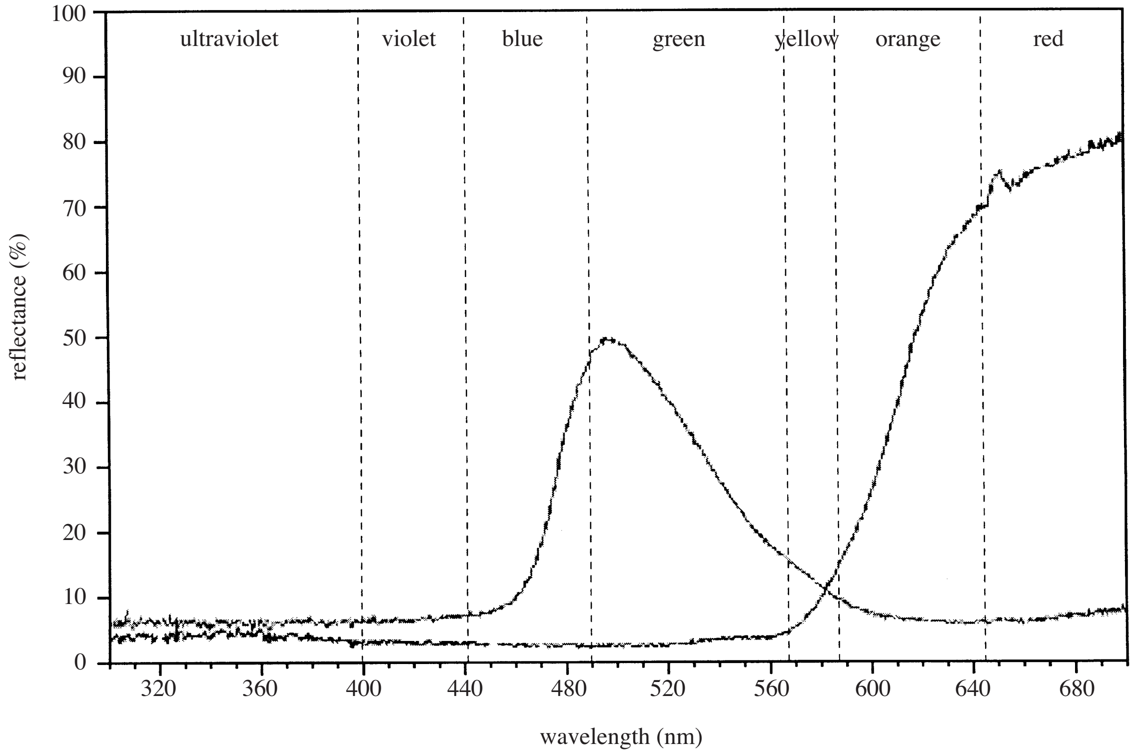

Figure 1. The reflectance spectra of the green and red paints used in the experiments, measured using a S1000-2LOS25U spectrometer (Ocean Optics, Dunedin, FL, USA) with a DH-2000 deuterium–halogen light source (215–1700 nm) and a reflectance probe (FCR-7UV200-1.5 × 100-2, Avantes, Eerbeek, The Netherlands).

may depend on the circumstances in which the stimulus is presented, suggesting that unlearned biases may differ between different food types.

In two separate experiments, we investigate the importance of food type in the unlearned colour preferences of birds. By using naive domestic chicks as predators of live seed bug nymphs and artificial 'fruits', we first investigate whether birds differ in their colour preferences according to whether the food item is fruit-like or an insect. We continue, in a second experiment, to investigate whether prey movement affects the unlearned colour preferences of domestic chicks.

2. MATERIAL AND METHODS

As predators in the experiments we used domestic chicks, *Gallus gallus domesticus*; male chicks were used in experiment 1 and female chicks were used in experiment 2 (permit D-nr. N60/00 by Stockholms djurförsöksetiska nämnd). Chickens originate from the Red Jungle Fowl (*Gallus gallus*), which is a generalist, and their diet includes several types of insects, seeds, fruits and plant parts found by pecking and scratching the ground (Collias & Collias 1967). Thus, chickens are suitable for this type of experiment, as they may potentially have retained mechanisms by which to discriminate insects from fruits. The birds arrived from the hatchery in batches of about 40 individuals that were less than 18 h old and had not yet eaten. Each batch was housed in a cage with a 100 cm × 55 cm steel-net floor and 20 cm high wooden sides. The roof of the cage was made partly of wood and partly of chicken wire. The cage was heated by a 60 W carbon light bulb, and the floor of the cage was covered with sawdust. All chicks were fed chick starter crumbs and water *ad libitum*. Throughout each experiment, birds in the batches were evenly divided among treatment groups.

In experiment 1 we used both live insect prey and artificial 'fruits' as food stimuli. In experiment 2 we used live and dead insect prey to investigate the effect of prey movement. The insect prey were fifth instar nymphs of the seed bug *Graptostethus servus* (Heteroptera: Lygaeidae), which are palatable to chicks (Gamberale & Tullberg 1998). These nymphs are *ca.* 5.3 ± 0.3 mm long (mean ± s.d.), and ovoid in shape. As fruit-like food items we used small pastry spheres (mix of flour, water and margarine) with a diameter of *ca.* 4 mm. Thus, the insects and 'fruits' were of the same area when viewed from above, but the insects were a little bit flatter and not completely round.

All prey items were painted either green or red using children's paint, Gouache tempera (Color and co, Lefranc and Bourgeois, Le Mans, France). A 1:1 mixture of 'brilliant orange' and 'primary red' was used for red, and a 1:1 mixture of 'leaf green' and 'emerald green' was used for green. We measured the reflectance spectra of the colours (figure 1). When dried, the paint formed an opaque shield of green or red, and the underlying colour of the item was not visible. On the insects the paint covered the whole dorsal side of the body except for the black head, legs and antennae.

The experiments took place in an arena with a 40 cm × 60 cm cardboard floor. The prey items were presented on a white background in two rectangular trays measuring 3.5 cm × 6 cm, which were lowered into two holes in the middle of the arena. The longer sides of the trays were facing each other, and the trays were 2 cm apart. The bottom of each tray was perforated with 14 holes of 2 mm in diameter. Underneath each tray was another similar tray fastened with masking tape. The birds could not see these bottom trays because they were situated at a level underneath the cardboard floor. As mentioned in § 1, odour may interact with prey coloration to affect birds' behaviour. Although *G. servus* nymphs are palatable to chicks (Gamberale & Tullberg 1998) and we have no evidence of them emitting any odour, we wanted to control for the possibility. Thus, in experiment 1 we placed five live *G. servus* nymphs in each bottom tray when 'fruits' were presented, and three nymphs in each bottom tray when insects were presented. In experiment 2, three live nymphs were always placed in the bottom tray irrespective of presentation type.

The experiments started on the birds' third day, when they were less than 60 h old, and were designed as preference tests between red and green prey items. At each trial, a bird was presented with two prey items, one of each colour and one in each tray. In experiment 1, a total of 81 birds were divided into two treatment groups receiving either insects ($n = 40$) or 'fruits' ($n = 41$). In experiment 2, we used a total of 66 birds, which were divided into two treatment groups receiving either live insects ($n = 33$) or dead insects ($n = 33$). Before the start of each experiment one bird of a batch was fed with mealworms, which made it inactive and not interested in feeding. This bird was used as a companion to the experimental birds during testing. A trial lasted for 5 min. If a bird did not attack the prey during a trial, the trial was repeated once each day until the bird responded, or for a total of four trials in experiment 1 and two trials in experiment 2. We measured the time to first attack and recorded the colour of the prey attacked first.

We compared the numbers of birds first attacking red and green prey items within treatments using goodness-of-fit tests and between treatments using contingency tables. The numbers of birds that did not attack a prey item during the experiments were compared between treatments using contingency tables. The time of first attack was compared between treatments using Mann–Whitney *U*-tests. For birds that did not attack any prey during the experiments, the times were set to 1200 s in experiment 1 and 600 s in experiment 2, which represent the total times that the birds had access to the prey.

3. RESULTS

(a) *Experiment 1*

Most birds attacked a food item; only 4 out of 40 birds presented with insects and 5 out of 41 birds presented with 'fruits' did not attack at any point during the 4 trials (figure 2). Whether the food was an insect or a 'fruit' did not seem to affect the birds' willingness to attack: the number of birds attacking did not differ significantly between the two prey types ($\chi^2 = 0.099$, d.f. = 1, not significant). Neither was there a significant difference in chick attack latencies between prey types (mean ± s.e.m. = 570 ± 58 s for 'fruits' and mean ± s.e.m. = 444 ± 60 s for insects; Mann–Whitney $U = 654$, $p = 0.117$).

In the birds presented with insects, there was a preference in the colour of the prey attacked first. A larger proportion of birds preferred the green insect to the red insect ($\chi^2 = 9.00$, d.f. = 1, $p < 0.01$). However, the birds presented with fruits did not show any significant colour preference ($\chi^2 = 0.444$, d.f. = 1, not significant). Thus, there was a difference between food type in the colour of the prey attacked first ($\chi^2 = 6.99$, d.f. = 1, $p < 0.01$) (figure 2).

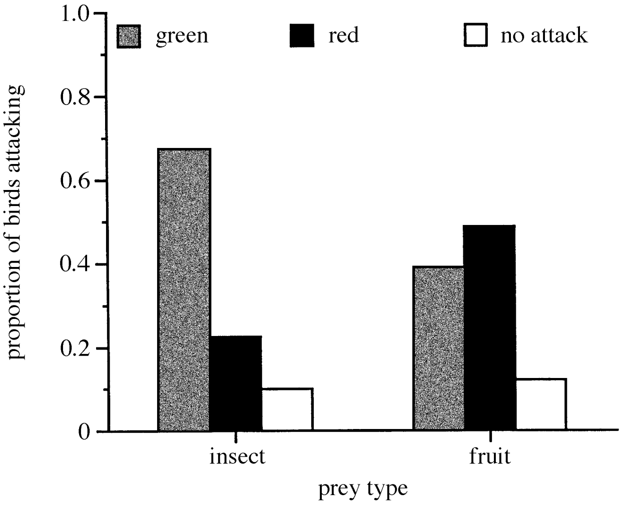

Figure 2. The proportions of naive chicks first attacking a green or a red food item, when the food items were either painted live *G. servus* nymphs or artificial 'fruits'. White bars indicate chicks that did not attack either prey item.

(b) *Experiment 2*

As in experiment 1, most birds attacked a prey item; only 5 out of 33 birds presented with dead insects and 7 out of 33 birds presented with live insects did not attack at any point during the two trials (figure 3); there was no significant difference between treatments ($\chi^2 = 0.41$, d.f. = 1, not significant). Whether the prey were dead or alive did not affect the birds' attack latencies significantly (mean ± s.e.m. = 306 ± 33 s for dead prey and mean ± s.e.m. = 259 ± 36 s for live prey; Mann–Whitney $U = 633$, not significant).

Prey movement did not seem to affect birds' colour preferences: birds did not differ between treatment groups in the colour of the prey attacked first ($\chi^2 = 0.467$, d.f. = 1, $p < 0.792$). Green prey were preferred to red prey both when the prey were alive ($\chi^2 = 12.46$, d.f. = 1, $p < 0.001$) and when they were dead ($\chi^2 = 11.57$, d.f. = 1, $p < 0.001$) (figure 3).

4. DISCUSSION

The birds' behaviour in experiment 1 showed that one type of food may elicit an unlearned colour preference or avoidance that is absent with another type of food. Young chicks did not show any difference in pecking preference between red and green when spherical fruit-like stimuli were used. However, when the prey were insects, green prey were strongly preferred to red prey.

It is known that the presence of an odour may alter chicks' behaviour towards prey (Marples & Roper 1996; Rowe & Guilford 1996, 1999; Roper & Marples 1997*b*; Lindström *et al.* 2001). However, it is unlikely that the results of our experiments can be attributed to differences in odour between treatments. This is because, first, the *G. servus* nymphs are undefended against being predated by birds, and do not emit any odour detectable by us, and, second, we controlled for the possibility that the bugs emitted an odour by presenting hidden bugs together with the non-insect prey, so that any odour would be present in both treatments. Neither does it seem that

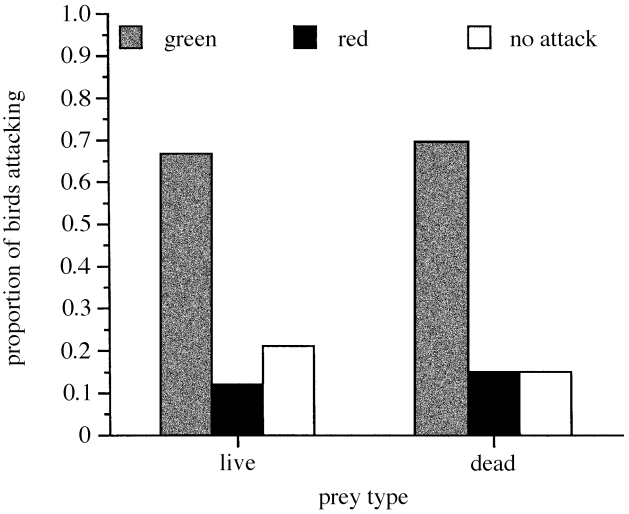

Figure 3. The proportions of naive chicks first attacking a green or a red food item, when the food items were either live or dead painted *G. servus* nymphs. White bars indicate chicks that did not attack either prey item.

prey movement in our experiments affected the chicks. Experiment 2 showed that chicks did not differ in behaviour when presented with live or dead prey: green prey were preferred to red prey in both treatments. The birds' general willingness to attack, expressed in terms of both the total number of birds attacking and the attack latencies, did not differ between prey types. This suggests that the birds did not find either prey type more aversive than the other. Thus, the degree of neophobia towards the 'fruits' and insects did not differ, only the relative preference of green and red prey.

Although quite similar, the two food types differed in some aspects. The most obvious differences were that the insects were not completely spherical, but the 'fruits' were, and that the insects had visible black legs, head and antennae. The presence of a black colour in association with red has been shown to affect the relative preference–aversion in chicks (Roper & Cook 1989). However, in their experiments, olive-green prey were preferred to completely black and completely red prey, but black-and-red striped and black-and-red bicoloured prey were more or less preferred to olive-green prey. Thus, if the black colour of the legs and antennae in our experiment affected the birds' preferences, then the effect was the opposite of that expected from Roper & Cook's (1989) results. Also, the shape of the food items may have affected the chicks, in which case items that are not completely spherical may elicit colour discrimination. However, this is not a totally convincing explanation, as it does not seem to be adaptive. Such behaviour would entail the exclusion of many small fruits that are not completely spherical in shape from the birds' diet. Still, the importance of the shape of the prey item in colour discrimination cannot be ruled out, as chicks' reactions to different shapes of prey have not been investigated. Another explanation for the difference in behaviour may be that the young chicks classified prey as insects, which in turn may have elicited a colour bias in attack probability. If so, it is uncertain what cues the birds may have used for such a classification. These experiments show that, for whatever reason, birds' unlearned reactions to a colour stimulus are dependent on the circumstances in which the stimulus is presented. Such an ability to discriminate between food types may be an adaptation to a situation where the same signal conveys different messages depending on whether the sender is an aposematic insect or a profitable fruit.

As mentioned in § 1, it seems that birds show unlearned foraging biases against particular colours often used in warning signals (Sillén-Tullberg 1985*a*,*b*; Roper & Cook 1989; Roper 1990; Schuler & Roper 1992). This relative avoidance of warning-coloured prey by inexperienced birds has been explained as an adaptation by predators not to attack unprofitable and sometimes directly harmful prey. However, whether birds show unlearned biases towards fruits of different colours is uncertain. Willson & Whelan (1990) propose a set of hypotheses that may explain fruit coloration. Among these hypotheses, some of the more intuitively appealing explanations are signal-related, with the colours promoting seed dispersal. Willson & Whelan (1990, and references therein) suggest that common fruit colours may reflect preferences in frugivores, may be more easily learned by birds, may work best for long-distance advertisements, may indicate fruit maturity or may be mimics of more profitable fruits. Although wild-caught frugivorous birds have sometimes been shown to prefer red fruit (McPherson 1988; Willson 1994; Puckey *et al.* 1996; Hartley *et al.* 2000), it is not clear whether this behaviour is innate or learned.

Thus, it is possible that birds do not inherently prefer fruits of certain colours, and it is also possible that it is not generally adaptive to show such behaviour. Many studies suggest that fruit properties such as conspicuousness, abundance or accessibility, size and caloric content may be as, or even more, important than fruit coloration *per se* for the fruit choices of birds (McPherson 1987; Willson *et al.* 1990; Willson & Comet 1993; Willson 1994; Giles & Lill 1999). Also, there are studies showing great individual variation in fruit colour preferences (Willson *et al.* 1990; Willson & Comet 1993; Willson 1994), which support this conclusion. Also, the behaviour of the chicks in the present experiment supports this idea, given that the chicks' lack of preference for either red or green fruit-like prey reflects their true innate behaviour. However, chickens are not specialist frugivores, and it is possible that different degrees of unlearned colour preferences exist in different types of predators, depending on their preferred food source. If this is the case, one would expect specialist insectivores to show greater aversion to prey items with a typical warning colour, such as red, than would omnivores or frugivores, and that specialist frugivores may have evolved some unlearned preferences toward common fruit colours.

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