

A Note on the Odour of Amyl Acetate in the Role of an Alerting Signal

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Introduction

Chapter 14 in this book (Barnea *et al.*, 2003) describes studies on the role of pyrazine (2-methoxy-3-isobutyl pyrazine), which can function as an alerting signal that chicks, recalling past experience of disagreeable events, could learn and associate with past events in nature. This alkaloid is widely distributed in toxic insects, such as monarch butterflies, burnet moths, and ladybird beetles, which are avoided by birds. The strong scent of amyl acetate, however, is characteristic of attractive fruit and flowers, and also appears to function as an alerting signal for frugivorous birds and frugivorous insects. In order to investigate the effect of amyl acetate on day-old chicks, we included them in similar experiments as those described by Barnea *et al.* (2003).

Materials

A total of 552 chicks were used for the combined experiments, of which 139 were employed for amyl acetate odour only. In both series of experiments, the birds were offered plain tap water or quinine-tainted tap water in red, yellow, green, indigo, or white drinking tubes associated with or without alerting odours. The amyl acetate was obtained from Sigma (Cat. amyl acetate 628-63-7, FW 130-2, hygroscopic $C_7H_{14}O_2$ diluted 1:1 in tap water) and the pyrazine from Pyrazine Specialities Inc., Georgia, USA. *Tables 15.1, 15.3 and Figure 15.1* follow those in Chapter 14 (Barnea *et al.*, 2003).

The distribution of amyl acetate (a mixture of isomers) in nature

Amyl acetate is well known for the melliferous smell it imparts to bananas. In the past, this oily extract of the fruit was used as a machine lubricant, and some reputable dictionaries still give 'banana oil' as a synonym for amyl acetate, although some of the other 200 volatiles identified from banana are also present in the extract (Simmonds, 1966; Nursten, 1970) and contribute to the odour of the fruit.

Unlike pyrazines, amyl acetate is rarely found in insects or in the foliage of plants, but has been recorded in the leaves of banana (M. Rothschild, *unpublished*). Isopentyl acetate was noted once in bees by Birch (1974) in a whole extract, and possibly in the heads of two species of New Zealand ant (Brophy, 1989). These two records contrast with the number of those for pyrazines which, for example, have been recorded from no less than 150 species of ant (Brophy, 1989).

Amyl acetate is a characteristic aroma of many fruits, such as pears, apples, oranges, and strawberries, and is recorded from the flowers of *Rosa*, *Cymbidium*, *Acrinidia*, *Cananga*, *Bubbia*, *Zygogynum* (Knudsen *et al.*, 1993) and *Musa velutina* (M. Rothschild, *unpublished*).

In the East Indies and Africa, wild bananas are pollinated by sunbirds, bats, and tree shrews (Proctor *et al.*, 1997), and in the New World's cultivated crops by humming birds. In Malaysia, various butterflies are attracted to the copious flow of nectar from the creamy white flowers of the banana. It is tempting to speculate that amyl acetate functions as an alerting signal for these pollinators, and also for the butterflies which feed on ripe fallen apples, plums, and pears in the UK.

Recorded experiments with the odour of amyl acetate and birds

Day-old, male domestic chicks (*Gallus gallus domesticus*) were given coloured beads, accompanied by an odour source of isoamyl acetate, by Burne & Rogers (1996), who recorded a definite dislike of the smell, the chicks responding by a head-shaking reaction on pecking at the bead.

In preliminary experiments carried out to prove that birds responded to odours of various kinds, Wenzel (1967, 1987), in a series of papers, recorded an increase in cardiac and respiration rates in different bird species, including domestic chickens, when exposed to various odours, including that of amyl acetate. Subsequent experiments have shown that the domestic pigeon can not only detect the odour of amyl acetate and butyl acetate (Walker *et al.*, 1979), but can discriminate different intensities of amyl acetate (Schumake *et al.*, 1969). Recording from bundles of the olfactory nerves of about a dozen species of birds, including the domestic pigeon (*Columba livia*), Tucker (1965) and Oley *et al.* (1975) showed that they all responded to the inspiration of the odour of amyl acetate. According to Wenzel (1971), the brown kiwi (*Apteryx australis mantelli*) refused food contaminated with the odour of amyl acetate.

Captive humming birds (*Colibri servcostis*) were found to respond to various odours, including that of amyl acetate, as discriminative stimuli (Ioalè & Papi, 1989). In nature, these birds are principally flower feeders and, according to Grant & Grant (1968), usually select those with little or no scent.

Relatively few experiments have been tried to pit odour against colour as attractants or repellents for birds. Roper & Marples (1997) noted that, when chicks were "trained to avoid quinine solution using a compound visual olfactory cue, the olfactory cue component overshadowed the visual one." On the other hand, Duncan & Slotnick (1985) noted that the food finding of domestic pigeons depended on visual, not olfactory, cues.

The odour of amyl acetate as an alerting signal

Plain tap water or quinine-tainted tap water was offered to the birds in red, yellow,

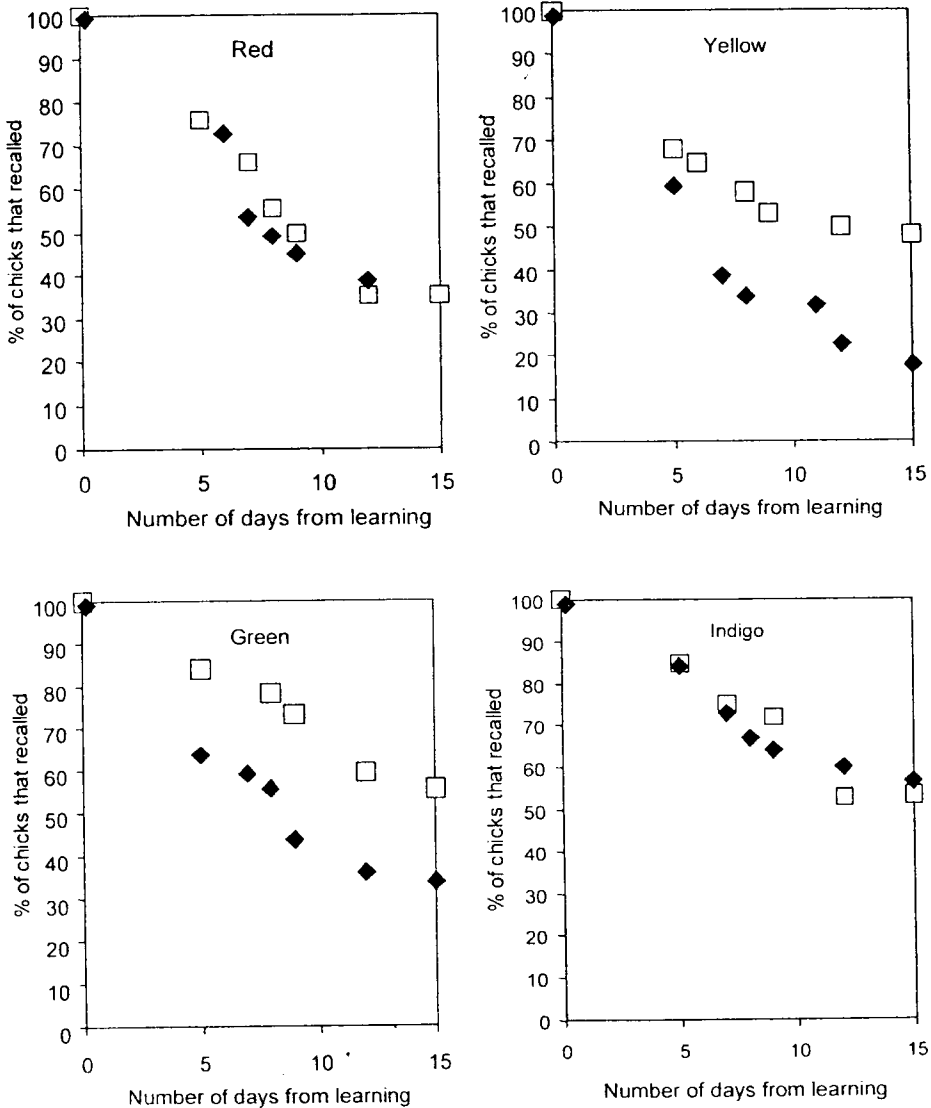


Figure 15.1. Percentage of chicks that recalled association between bitter water and the colour of the water tube, with (□) or without (◆) amyl acetate odour, as a function of the number of days from learning.

green, and indigo coloured drinking tubes, with and without the presence of alerting odours. A synopsis of the results of the comparison with amyl acetate and the pyrazine is given in *Table 15.1*. The experimental design, statistical analysis, general frame of the experiments, and the pyrazine results are described in Chapter 14 (Barnea *et al.*, 2003).

Without either of the alerting signals, there was no significant improvement in the recall or learning process associated with bitter water, except in the case of the indigo tube, which significantly improved recall (*Table 15.1*, *Figure 15.1*) without the addition of an alerting signal. When the odour of amyl acetate was added, recall was

Table 15.1. Reaction of chicks to quinine-tainted water with or without alerting odours offered in coloured drinking tubes.

Alerting odour added	Recall improved	Learning period shortened
amyl acetate	green and yellow tubes	red, green, and indigo tubes
2-methoxy-3-isobutyl pyrazine	red, yellow, and green tubes	red and indigo tubes
no alerting odours added	indigo tube only	no effect

Table 15.2. Chi-square values for comparisons between different recall curves (see *Figure 15.1*).

Colour of water tube associated with the bitter taste	$\chi^2_{df=1}$ for comparison of the recall curves for this association and the same with amyl acetate odour
red	not significant
yellow	6.95 ($P = 0.008$)
green	5.93 ($P = 0.015$)
indigo	not significant

Table 15.3. Mean (\pm s.e.) to reach the learning criterion (see text) for an association between bitter taste and a colour with/without pyrazine or amyl acetate odour (figures in italics are taken from Barnea *et al.*, 2003).

Colour	<i>Without odour</i>	<i>With pyrazine odour</i>	<i>With amyl acetate odour</i>
red	<i>10.0 \pm 0.29 (n = 47)</i>	<i>8.8 \pm 0.17 (n = 54)</i>	<i>8.8 \pm 0.21 (n = 40)</i>
yellow	<i>9.0 \pm 0.21 (n = 44)</i>	<i>8.6 \pm 0.15 (n = 43)</i>	<i>8.6 \pm 0.16 (n = 40)</i>
green	<i>9.7 \pm 0.26 (n = 50)</i>	<i>9.0 \pm 0.20 (n = 49)</i>	<i>8.6 \pm 0.18 (n = 43)</i>
indigo	<i>9.7 \pm 0.28 (n = 45)</i>	<i>8.8 \pm 0.15 (n = 61)</i>	<i>8.8 \pm 0.19 (n = 36)</i>

significantly improved if associated with green and yellow tubes, but not red or indigo (*Tables 15.1 and 15.2*), while the learning process significantly improved with red, green, and indigo tubes (*Table 15.3*). When the odour of pyrazine was added, recall was significantly improved with red, yellow, and green tubes, and the learning process with red and indigo tubes, but not green or yellow tubes (*Tables 15.1 and 15.3*). If bitter water was presented to birds in red and indigo tubes; in the presence of the odour of amyl acetate or the pyrazine, the learning process was shortened in both cases ($F = 7.96$, $df = 2$, $P = 0.0005$ and $F = 6.71$, $df = 2$, $P = 0.0017$ respectively). If only yellow tubes were used with the odour of amyl acetate or the pyrazine, no significant difference in the learning process was recorded. If only green drinking tubes were employed with both odours, only amyl acetate elicited a significant shortening in the learning process ($F = 6.42$, $df = 2$, $P = 0.002$) (*Tables 15.1 and 15.3*).

These experiments demonstrate certain differences in the reaction of the birds to the coloured tubes associated with the two distinctive alerting signals.

This was perhaps not surprising, since the distribution of the two signals in the animal and plant kingdoms is markedly different, but it is interesting to find that, in our experiments, alerting signals were usually required in order to initiate a specific response to a colour. Marples *et al.* (1994) previously showed that birds trained to avoid toxic ladybird beetles (*Coccinella septempunctata*) needed both their aposematic coloration and the alerting odour of a pyrazine to be presented simultaneously in order

to respond as anticipated and show avoidance. Segregated, these cues did not initiate avoidance. This also suggests that alerting signals may exert a subtle influence which is not, in all cases, limited to the arousal of equitable interest, or the anticipation of pleasure or pain.

Discussion

The comparison between the reaction of male, day-old chicks to the pyrazine and amyl acetate, together with their respective distributions in nature, suggests that amyl acetate is often an alerting signal for the presence of edible fruit, or the nectar in flowers – promising an agreeable experience – while in insects, pyrazine frequently functions as a warning signal for a wide range of many toxic or dangerous species of animals and plants. Both substances can, as circumstances dictate, change roles.

In our experiments, amyl acetate was linked with a disagreeable experience, to which signal the chicks responded when it was associated with specific colours, which were sometimes different (see *Table 15.1*) from those which stimulated recall and learning when linked to the pyrazine.

The known distribution of these two alerting signals is, in a sense, inevitably distorted by the interests of the biologists concerned. They are usually found as side effects only of some central theme, and do not form the main objective of the investigation. In the case of pyrazines, trace amounts are so widely distributed in plants, where they enhance and intensify the flavour of other components, that they are generally overlooked, and only noted when their concentrations are greatly increased and function as warning signals, for example in the foliage of *Asclepias* and *Urtica*. Both pyrazines and amyl acetate are often seasonal, and are then consequently not infrequently overlooked. Thus, the nettle (*Urtica urens*) is devoid of the pyrazine until the summer (M. Rothschild, *unpublished*), and amyl acetate is only present when fruit ripens and seeds are ready for dispersal.

We now require experiments in which the chicks are offered water adulterated with an attractant rather than a repellent. It is probable this will reinforce the difference noted here in the birds' reaction when the two alerting signals were linked to different coloured tubes.

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Postscript

The owl butterfly (*Caligo memnon*) was present in numbers free flying in March 1999 in Mr Clive Farrell's Butterfly House at Stratford, UK. They were feeding steadily at 10 a.m. on over-ripe, peeled bananas placed on a table. Transferred to a sheet of cotton wool with a small puddle of 2% amyl acetate in water on the surface, they quickly

extended their proboscis and began to imbibe the fluid. Several continued to do so without stopping for 5–32 minutes. Some individuals paused and prodded round the area with the proboscis and either resumed feeding or flew off. They were not attracted to the area from a distance. The behaviour of these butterflies suggested that amyl acetate signals an attraction, and stimulates a linked feeding and searching response.

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