Taste-rejection behaviour by predators can promote variability in prey defences

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The evolution and maintenance of toxicity in a prey population is a challenge to evolutionary biologists if the investment in toxin does not benefit the individual. Recent experiments suggest that taste-rejection behaviour enables predators to selectively ingest less toxic individuals, which could stabilize investment in defences. However, we currently do not know if taste rejection of defended prey is accurate across different contexts, and that prey always benefit according to their investment. Using avian predators, we show that the rejection probability does not solely depend on the investment in defence by an individual, but also on the investment by other individuals in the same population. Therefore, taste rejection by predators could lead to destabilization in the investment in defences, and allow variability in prey defences to exist.

Keywords: domestic chick; insect defence; crypsis; automimicry; avian taste; aposematism

1. INTRODUCTION

Many prey species possess toxins to ward off predatory attacks. Toxic prey are often aposematic, advertising their toxins using warning signals (Cott 1940; Bowers 1992), but toxic cryptic species also exist (e.g. Wheeler *et al.* 1970). Understanding how chemical defences evolve and are maintained in a population is a major challenge for evolutionary biologists (Guilford 1994; Speed *et al.* 2006; Skelhorn & Ruxton 2008). Investing in defences can be costly, for example, in terms of impaired growth rates (Bowers 1992). Therefore, some individuals may invest less in toxins, and enjoy a selective advantage from being indistinguishable from more toxic conspecifics. So, how is toxicity maintained? This has been called the problem of ‘automimicry’ in the context of aposematism (Brower *et al.* 1967; Guilford 1994), although the same problem arises in cryptic populations (Skelhorn & Rowe 2006b; Speed *et al.* 2006).

Investment in defences could be stabilized if toxicity correlates with individual survival, for example, if toxins are tasted by predators upon attack, and prey are rejected according to their perceived defence level (Wiklund & Järvi 1982; Sillén-Tullberg 1985). Most naturally occurring toxins taste bitter as animals have evolved bitter taste receptors to detect toxins in food (Glendinning 2007). While distastefulness in insects may not always correlate with toxicity levels (e.g.

Marples 1993), we also know that birds can selectively predate butterflies, preferring those containing lower levels of bitter-tasting toxins (Smith 1979; Brower & Calvert 1985). Recent laboratory experiments have shown that birds can taste the difference between defended and undefended prey in both aposematic and cryptic populations (Skelhorn & Rowe 2006b; Halpin *et al.* 2008a,b). However, if birds can accurately taste-reject prey that invest in different levels of toxins (Gamberale-Stille & Guilford 2004; Skelhorn & Rowe 2005), this leaves a different problem of trying to explain the widespread existence of variation in toxicity in prey populations (e.g. Brower *et al.* 1967; Ruxton & Speed 2006).

One explanation is that predators may not always accurately taste-reject prey. For example, it might be costly to spend time discriminating among variably defended prey (Ruxton & Speed 2006), or variability may be confusing for predators. Our experiment tested whether predators always taste-reject prey according to their toxicity, and whether we can assume that survival depends upon individual investment (Speed *et al.* 2006). Specifically, we compared how mildly and moderately defended prey were rejected by predators when they were the only defended prey type in a cryptic prey population compared with when they occurred together.

2. MATERIAL AND METHODS

Forty-eight experimental domestic chicks (*Gallus gallus domesticus*) of mixed sex and 12 ‘buddy chicks’ (see below) were hatched and housed in the laboratory (Halpin *et al.* 2008b). Undefended, mildly defended and moderately defended prey were chick crumbs sprayed with either water, 1 per cent or 4 per cent quinine sulphate solution, respectively. Quinine is a bitter-tasting toxin, and is aversive to chicks at these concentrations (e.g. Halpin *et al.* 2008a). Once dry, crumbs were sprayed with green or purple food dye (Halpin *et al.* 2008b).

The experimental arena was a cage (100 × 50 × 50 cm) with a mesh barrier placed 25 cm from one end which housed two buddy chicks to prevent social isolation. The floor was laminated green or purple paper, which matched the crumb coloration. On days 1 and 2 post-hatch, experimental chicks were trained (initially in groups of three, then in pairs and finally individually to eat uncoloured chick crumbs in the arena. Half the chicks were trained and tested on a purple background and the other half on a green background (Halpin *et al.* 2008b). A grid drawn on each background allowed us to randomly place the crumbs and identify which were attacked. On day 3, chicks were food-deprived for 1.5 h and placed individually in the arena for the experimental trials. Each chick was given 20 undefended prey, and either 20 mildly defended prey (mildly defended group, $N = 14$), 20 moderately defended prey (moderately defended group, $N = 11$) or 10 mildly defended and 10 moderately defended prey (mixed defence group, $N = 11$). All prey were cryptic (i.e. the same colour as the background), and were visually indistinguishable (see §3). The chicks were allowed to attack 16 crumbs in each trial. We recorded which prey were attacked and if they were eaten or rejected. Each chick received two trials per day for three consecutive days.

There was no effect of prey coloration in any of our analyses, so data were pooled across colour treatments. We used a generalized linear model (GLM) to analyse rejection probabilities, with the mean level of quinine of the defended prey as a continuous variable (i.e. 1% for the mildly defended group, 2.5% for the mixed defence group and 4% for the moderately defended group) and defence type (undefended or defended) as a repeated measure. This method enabled us to test whether the rejection probability for prey in the mixed defence group was not only different from, but also intermediate to the mildly and moderately defended groups (Skelhorn & Rowe 2006b). We also used a generalized estimating equation (GEE) to analyse changes in taste-rejection behaviour across trials.

3. RESULTS

We calculated the mean taste-rejection probability for defended and undefended prey across all trials. As

Figure 1. The mean ($\pm$ s.e.) rejection probability of undefended (white bars), mildly defended (grey bars) and moderately defended prey (black bars) in the three experimental treatments.

expected, defended prey were rejected more often than undefended prey ($F_{1,33} = 55.79$, $p < 0.01$; figure 1). The rejection rates of both defended and undefended prey were positively correlated with the mean defence level of the population ($F_{1,33} = 6.601$, $p < 0.01$), with no significant interaction ($F_{1,33} = 0.149$, $p > 0.05$). Defended prey in the moderately defended group were rejected significantly more than defended prey in the mildly defended group (t -test: $t = 3.23$, $p < 0.05$, d.f. = 23), but there was no significant difference in the rejection of mildly and moderately defended prey in the mixed defence group (paired t -test: $t = 0.29$, $p > 0.05$, d.f. = 10; figure 1).

Taste-rejection behaviour on each prey type also changed across our six trials (figure 2). We found significant interactions between trial and group on taste rejection for all prey types (GEE; undefended: Wald $\chi^2 = 44.88$, $p < 0.01$; mildly defended: Wald $\chi^2 = 13.82$, $p < 0.05$; moderately defended: Wald $\chi^2 = 13.90$, $p < 0.05$; figure 2). We subsequently compared rejection probabilities between trials 1 and 6 using paired t -tests. For undefended prey, we found a near significant decrease in rejection rates in the mildly defended group ($t = 2.05$, $p = 0.06$), an increase in the moderately defended group ($t = 5.56$, $p < 0.01$), but no change in the mixed defence group ($t = 0.21$, $p > 0.05$). For the mildly defended prey, rejection probability decreased in the mildly defended group ($t = 3.32$, $p < 0.01$), but there was no change in the mixed defence group ($t = 0.46$, $p > 0.05$). We also found that the rejection of moderately defended prey increased in the moderately defended group ($t = 3.23$, $p < 0.01$), with no change in the mixed defence group ($t = 0.14$, $p > 0.05$). Overall, chicks in the mildly and moderately defended groups were changing their rejection behaviour towards defended and undefended prey during the experiment, while there were no changes in the mixed defence group.

The differences in rejection behaviour were based upon the taste of the prey and not on any visual differences. We compared the probability of attack for each prey type to what would be expected at random (i.e. 0.4, as 16 of 40 crumbs were attacked in total). This was not significantly different from random for any prey type in any group (one-sample t -tests: $t < 2.01$, $p > 0.05$ for all comparisons).

Figure 2. The mean ($\pm$ s.e.) rejection probability across trials for (a) undefended, (b) mildly defended and (c) moderately defended prey in the mildly defended (squares), mixed defence (triangles) and moderately defended (circles) groups.

4. DISCUSSION

Our data show that the probability of being taste-rejected by a predator does not solely depend upon an individual's toxicity. Birds did not discriminate between the two defended prey types when they occurred together in a mixed population, meaning that the mildly defended prey benefited from the investment in defence of the moderately defended prey. This indiscriminate behaviour would lead to the destabilization in the investment in toxins. Therefore, the effectiveness of a defence appears to depend upon the structure of the prey population, and selection from taste-rejection behaviour can be variable, allowing multiple defence levels to persist (Ruxton & Speed 2006).

One explanation for the birds' inability to discriminate between the mildly and moderately defended prey in the mixed population is an inability to distinguish between these two levels of quinine. However, this is not the case: birds discriminated between mildly and moderately defended prey when they were both conspicuously coloured red and visually distinct from cryptic undefended prey (Skelhorn & Rowe 2006b). However, it could be that they are unable to discriminate among three different levels of defence within a population, leading to discrimination error and allowing less defended individuals to benefit and persist in the population.

Alternatively, taste rejection may depend upon how animals learn to associate taste with toxicity (Skelhorn & Rowe 2006b, 2010). Changes in rejection probabilities were evident across trials in the mildly and moderately defended groups, suggesting that birds were altering their taste-rejection strategies in response to the post-ingestive effects of quinine. When the effects were mild, chicks decreased their rejection rates, while at moderate concentrations of quinine, birds rejected more prey in an attempt to achieve an acceptable ingestion rate. In the mixed defence group, chicks did not change their rejection probabilities, and may have found it difficult to learn to associate multiple quinine concentrations with different tastes (Yearsley *et al.* 2006). In the wild, other factors (e.g. encounter rate) may enable predators to learn to reject distasteful prey according to their toxicity; however, we need to know more about the taste-rejection behaviour of wild predators to fully predict the impact on natural populations.

Understanding the taste-rejection strategies employed by predators is crucial for understanding the evolutionary dynamics of distasteful toxins (Guilford 1994; Ruxton & Speed 2006; Speed *et al.* 2006). In contrast to other recent experiments (Gamberale-Stille & Guilford 2004; Skelhorn & Rowe, 2006a,b; Halpin *et al.* 2008a), we find that taste-rejection behaviour does not always give benefits to prey according to their toxicity. Therefore, we cannot assume that taste-rejection behaviour will always increase the stability of defences, and instead could provide opposing selection pressures that allow automimics to be maintained in the population. Although other factors might also affect the relative costs and benefits of investment in defences, for example, the costs of storing toxins or life-history trade-offs in prey (Ruxton & Speed 2006), we provide the first evidence that taste-rejection behaviour can exert variable selection pressures on investment in defences.

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