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Original Article

Educated predators make strategic decisions to eat defended prey according to their toxin content

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Animals often eat foods containing toxins to benefit from the nutrients that they contain. Understanding how animals balance the costs of eating toxins with the benefits of gaining nutrients is important for understanding the evolution of antipredator defenses, particularly aposematism and mimicry. In this study, we tested whether predators could learn to use color signals to make strategic decisions about when to include prey that varied in their toxin content in their diets. We gave European starlings (*Sturnus vulgaris*) daily sessions of sequentially presented mealworms (*Tenebrio molitor*). There were 3 types of mealworm which were made discriminable using color signals: undefended mealworms injected with water, mildly defended mealworms injected with 1% quinine solution, and moderately defended mealworms injected with 3% quinine solution. Birds learned to eat more undefended than defended prey and more mildly than moderately defended prey. Crucially, when we manipulated the birds' energetic states using food restriction, we found that they increased the number of defended prey that they ate but maintained their relative preferences. Birds made state-dependent decisions based upon their knowledge of the amount of toxin prey contained and their current energetic need. Our results provide novel insights into the evolution of aposematic signals and also demonstrate that we may need to develop new models of the evolution of mimicry based on the state-dependent behavior of predators. Our data also have broader implications for the study of nutrient–toxin trade-offs across a range of different ecological scenarios. *Key words:* Müllerian mimicry, predation, state-dependent foraging, toxin regulation, warning coloration. *[Behav Ecol 23:418–424 (2012)]*

INTRODUCTION

Wild animals encounter a variety of different foods, many of which will be defended with toxins. For example, pollinators feed from flowers that have alkaloids in their nectar (e.g., Adler 2000), herbivores graze on plants that contain secondary metabolites (e.g., Marsh et al. 2006), frugivores consume fruit that contains high tannin levels (e.g., Cipollini and Douglas 1997), and predators attack and eat prey that contain a variety of different toxins (e.g., Brower 1984). The ability to eat foods containing toxins allows animals to access the nutrients that they contain. Consequently, animals have evolved a range of different adaptations, both physiological and behavioral (e.g., De Souza et al. 2002; Dearing et al. 2005; Hanifin et al. 2008) that enable them to ingest toxic foods. An important adaptation is the ability for animals to be able to learn about the toxicity of different foods in order to carefully control their intake of toxins, as well as their intake of nutrients.

One area in evolutionary biology where learning about toxicity is important is the evolution of aposematism and mimicry. Toxic prey often advertise their defenses to predators using conspicuous coloration, a strategy known as "aposematism" (Poulton 1890). Naïve predators readily associate warning coloration with toxicity, and the speed with which they learn to avoid aposematic prey depends on both the conspicuousness of the signal (e.g., Gittleman and Harvey 1980) and the nature of the chemical defense (e.g., Skelhorn and Rowe 2006a, 2006b). Avoidance learning has also underpinned the study of Müllerian mimicry, which occurs when 2 or more aposematic species share the same warning pattern (Müller 1879). Traditionally, Müllerian mimics are thought to benefit through shared predator education; if predators eat a fixed number of prey before they learn to associate the coloration with toxicity and avoid warningly colored prey, then co-mimics with the same color pattern would mutually benefit from sharing this cost (Müller 1879). However, more recent theoretical treatment suggests that when co-mimics differ in their toxicity, the less defended co-mimic might benefit at the expense of the more defended co-mimic, a phenomenon referred to as "quasi-Batesian" mimicry (Speed 1993). Studies investigating the evolutionary dynamics of Müllerian mimicry have focused on the speed of aversion learning in naïve predators, and how many prey are killed during the learning process (e.g., Rowe et al. 2004; Lindström et al. 2006; Rowland et al. 2007). This means that we know very little about the selection pressures acting on models and mimics once learning is completed.

An alternative approach is to consider the role of "educated predators," that is, those that have learned about the nutritional and toxic properties of the prey that they encounter and ingest them strategically based on what they know about the prey and their current physiological state. We know that

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predators continue to eat toxic prey in the wild (e.g., Chai 1986; Pinheiro 2003), and laboratory experiments confirm that educated predators learn to use visual signals to ingest toxic prey according to their current need for nutrients (Barnett et al. 2007), as well as their toxin burden (Skelhorn and Rowe 2007). These studies support the basic assumptions of several recent state-dependent mathematical models of Müllerian mimicry that predators can learn to use visual signals to trade-off the ingestion of nutrients and toxins (Kokko et al. 2003; Sherratt 2003; Sherratt, Speed, et al. 2004). However, these experiments on educated predators have used only one toxic prey type with a single visual signal, and currently, we do not know whether predators can learn to associate relative or absolute toxicity of multiple prey types with their visual appearance, or how physiological state influences the level of discrimination among differentially toxic prey. Answers to these questions are important if we are to construct realistic state-dependent models describing the impact of educated predators on the evolutionary dynamics of aposematism and mimicry.

Here, we investigate the effects of state on predators' decisions to eat differentially toxic prey using European starlings (*Sturnus vulgaris*) foraging on quinine-injected mealworms in the laboratory (see also Barnett et al. 2007; Skelhorn and Rowe 2007; Skelhorn and Rowe 2010). We predicted that birds would be able to learn to use color signals to discriminate between prey containing different amounts of toxin and once educated, preferentially ingest the least defended prey type. In addition, we also predicted that the foraging decisions of the educated birds would be based upon their current physiological state: Increasing birds' energetic needs should increase their willingness to ingest defended prey.

MATERIALS AND METHODS

Subjects and housing

Six wild-caught male European starlings (*S. vulgaris*) were captured under license from English Nature (License Nos. 19991381 and 20001512) and housed individually in wire mesh cages measuring 75 × 45 × 45 cm equipped with 2 dowel perches and 2 water bottles. Bathing water was provided regularly. The birds were subject to a 14:10 h light:dark cycle under full spectrum fluorescent light. During the training phase, birds received ad libitum pheasant breeder starter pellets, but food was restricted during the mass manipulation phase (see below for details). Birds were caught every morning and weighed to the nearest 0.1 g. At the end of the experiment, birds were returned to a free-flight room before being released back to the wild at the site from where they were captured (7–9 months after capture).

Prey

Live mealworm larvae (*Tenebrio molitor*) measuring approximately 20 mm in length were used as prey. We manipulated their toxicity by injecting them intraorally with different solutions: undefended prey were injected with 0.02 ml of water, mildly defended prey were injected with 0.02 ml of 1% quinine sulphate solution, and moderately defended prey were injected with 0.02 ml of 3% quinine sulphate solution. The 3 prey types were made visually distinct using color cues (see below).

Training phase

Birds were initially trained to eat mealworms from Petri dishes (38 mm diameter) in their home cages. Before each training session, birds were food deprived for 2 h. Five minutes before the start of a session, their home cage was positioned behind a curtain so that they were visually isolated from other birds and the experimenter. During training sessions, birds' behavior was observed and recorded using a video camera connected to a monitor located in the room. Each day, birds received a session comprised of 12 sequentially presented mealworms each placed individually in a clear Petri dish on the floor of the cage. In this phase, the mealworms were not injected with solution and were presented on a white background. A mealworm was presented every 3 min, and the bird had 1 min to decide whether or not to attack and eat it. If the mealworm was not attacked, the Petri dish and the uneaten mealworm were removed after 1 min. However, if the mealworm was eaten, the empty dish was removed immediately.

Once a bird had consumed 5 mealworms in succession, training proceeded to the next stage where the birds learned to discriminate among the undefended, mildly defended, and moderately defended prey. The 3 prey types were each associated with a distinct color cue (green, orange, or purple) in the form of a colored disc of paper placed underneath the Petri dish containing the mealworm. Since there were 6 birds and 6 possible color combinations for the 3 prey types, color associations were counterbalanced across birds, and each bird had its own unique combination. Each daily session now consisted of 18 sequentially presented prey, 6 of each prey type. In order to ensure that birds readily learned the difference between the 3 prey types, each was presented in a block of 6 presentations within a session. Undefended prey were always presented in the first 6 presentations, with mildly and moderately defended prey being presented in the 2 following blocks of 6 trials, with equal probability. We recorded how many prey were attacked and eaten. Birds were trained until they had acquired the discrimination and had established a stable preference for the 3 prey types, which took individuals between 16 and 25 sessions. We defined acquisition as being when a bird's choices from the previous 3 days significantly departed from chance, and they ate more undefended prey than defended prey and also more mildly defended than moderately defended prey (mean ± standard error of total numbers of mealworms consumed during the final 3 days of training: undefended = 15.2 ± 0.65, mildly defended = 7.8 ± 1.05, moderately defended = 3 ± 0.26; all $\chi^2 > 7.462$, all $P < 0.05$, degrees of freedom [df] = 2). Upon achieving the criterion, we considered the birds to be educated predators, and began the mass manipulation phase.

Mass manipulation phase

We began manipulating birds' masses in order to investigate how their energetic state influenced their decisions to incorporate toxic prey into their diets. In the first phase (the mass loss phase), we restricted the amount of food birds received in order to reduce their masses. In the second phase (the mass gain phase), we gradually increased their daily food intake and allowed birds' masses to increase. Throughout both phases, birds continued to receive daily experimental sessions of the 3 prey types using a similar method of presentation. However, in this phase, the prey types were no longer presented in 3 blocks but were pseudorandomized across a trial. In order to balance prey types across a session, 2 of each prey type occurred in a random sequence in each third of the session.

In the mass loss phase, we aimed to reduce the birds' masses by approximately 0.5 g per day by restricting the daily amount of food each bird received. We calculated each bird's free-feeding mass (FFM), which was its mean mass during the last 5 days of the training phase. We then reduced the birds' masses until they ate all 18 prey that were presented in a session (the masses at which a bird first ate all 18 prey ranged from 91.8 to 98.5% of their FFM; see Table 1). Since we were

Table 1
The FFM, the change in mass in each phase, and the length of each phase for each bird

Bird	FFM (g)	Mass loss phase		Mass gain phase	
		Mass range (g)	Number of sessions	Mass range (g)	Number of sessions
19	74.4	75.1–71.0	10	72.9–76.0	12
20	75.4	74.8–70.1	8	72.3–75.1	12
21	77.9	77.9–75.9	10	77.6–80.7	7
39	79.7	80.5–73.2	6	74.4–75.1	4
48	74.4	76.7–72.5	14	72.6–76.0	9
86	79.0	78.3–72.5	6	72.9–73.4	5

interested in comparing birds' decisions on mildly and moderately defended prey, once birds included all prey in their diets and showed no variation in behavior, there was no need to reduce their masses any further. The length of the mass loss phase ranged from between 6 and 14 days across birds (for details for each bird, see Table 1). Birds were then maintained at this mass for 2 days, before we started to slowly increase the amount of pheasant breeder pellets that they were receiving, with the aim of increasing their masses by approximately 0.5 g each day. However, despite birds' masses slowly increasing, they did not immediately begin to exclude defended prey from their diets. Therefore, when we compared the masses between the first and last day that a bird ate all 18 prey, we found that birds were significantly heavier on the last day compared with the first day (paired *t*-test; $t = 3.031$, df $= 5$, $P < 0.05$). During this period, birds ate almost all the prey that were presented to them, making it impossible to analyze the data with respect to our hypotheses. Therefore, we did not analyze this part of the mass manipulation phase. Instead, we analyzed the data from the last day on which a bird ate all 18 prey to the end of the experiment and called this the mass gain phase. The mass gain phase also varied in length among birds, ranging between 4 and 12 days. Throughout all trials, we recorded whether prey were eaten or not and also the latencies to attack prey which were eaten (measured from the start of a presentation to when the bird touched the prey). All manipulations were conducted under ethical approval from Newcastle University and complied with Association for the Study of Animal Behaviour Ethical Guidelines.

Simultaneous choices

At the end of the mass manipulation phase, we gave the birds a series of simultaneous choice sessions over 9 days to test what cues they were using to discriminate amongst the 3 prey types. These sessions followed the same basic methodology, except that instead of a single mealworm being presented every 3 min, 2 mealworms were presented simultaneously, and the birds were allowed to attack and eat only one of them. They were given one of the following pairings: undefended and mildly defended prey; undefended and moderately defended prey; or mildly defended and moderately defended prey. There were 3 different treatments for each pairing. In the "Color–quinine" treatment, all prey types were presented in the same manner as during the mass manipulation phase, that is, they were injected with water or quinine solution as appropriate, and were associated with their color cues. In the Color-only treatment, we removed quinine, and defended prey were also injected with water; this tested whether or not birds had learned to use the color cues to discriminate between prey. In the Quinine-only treatment, we removed the color cues and presented all prey on a white background. Prey were injected with quinine and water as appropriate in order to test whether birds could discriminate between prey types without their associated color cues. Birds were given a single session on each day, and the order of the 9 sessions was randomized across birds.

Statistical analyses

Birds almost invariably ate all the undefended prey that were presented to them, and we therefore restricted our analyses to the 2 defended prey types. We analyzed the mass loss and mass gain phases separately due to the significant differences in mass ranges between these 2 phases (see above). For each phase, we tested whether decreases in energetic state (%FFM) increased the amount of toxin eaten and the numbers of mildly and moderately defended prey eaten in a session. For these analyses, we calculated the proportion of each prey type presented that was eaten in each session for all subjects. We then arcsine square root transformed the proportional data to allow us to perform parametric Generalized Linear Models (GLMs) on the proportion of prey eaten in a given session with %FFM as a covariate and subject as a random factor.

We also investigated if birds stopped eating moderately defended prey earlier in a session compared with mildly defended prey, as would be expected as birds' toxin burdens and masses increased during the session. In each session, we attributed a positional value to each presented prey, from 1 for the first prey to 18 for the last prey. We then took the positional values for defended prey that were eaten and calculated the mean positional score for each defended prey type. In a small number of sessions, birds only ate one type of defended prey, and these sessions were removed for this particular analysis. Again, we compared the mean positional scores for the 2 defended prey types using a GLM with %FFM as a covariate and subject as a random factor.

Finally, we also tested whether the time birds took to make their decisions to eat prey was affected by their nutritional state or the quinine content of the prey. To test this, we compared the latencies to attack each prey type from the first 2 days and the last 2 days of the mass gain and the mass loss phases. We used the mean latency data from 2 days in order to ensure that we had measurements for each prey type (in a small number of sessions, birds did not eat any of one of the defended prey types). We used a repeated measures analysis of variance, with prey type and the time in the phase (first or last 2 days) as within-subject factors.

RESULTS

Total amount of toxin ingested

We calculated the amount of quinine injected into mildly and moderately defended mealworms and then totaled the scores for each session. The total amount of toxin eaten increased with decreasing mass in both phases (mass loss phase: $F_{1,47} = 43.77$, $P < 0.001$; mass gain phase: $F_{1,41} = 9.49$, $P < 0.01$; see Figure 1).

Numbers of mildly and moderately defended prey eaten

In both the mass loss and the mass gain phases, birds ate more defended prey as their masses decreased (mass loss phase: $F_{1,95} = 57.93$, $P < 0.001$; mass gain phase: $F_{1,83} = 12.65$, $P = 0.001$; see Figure 2) and ate more of the mildly defended prey than the moderately defended prey (mass loss phase: $F_{1,95} = 52.45$, $P < 0.001$; mass gain phase: $F_{1,83} = 21.78$, $P = 0.001$; see Figure 2).

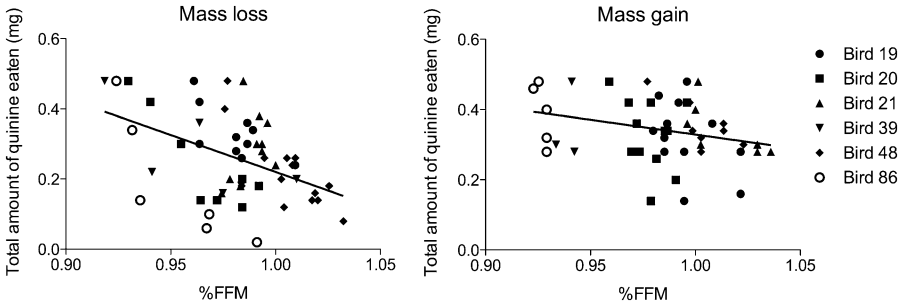

Figure 1
Decline in the total amount of toxin eaten in a session as the percentage FFM increased in the mass loss and the mass gain phases. Regression lines are shown for the untransformed pooled data.

Position in a session when mildly and moderately defended prey were eaten

The difference in the numbers of mildly and moderately defended prey eaten could have resulted from birds stopping eating moderately defended prey earlier in a session (either in response to their toxin burden increasing or their energetic state improving). Therefore, we tested this using the mean position in the session at which birds took the decision to eat prey of each defended prey type. In both phases, mildly defended prey were eaten for longer in a session (i.e., they had a greater mean positional values) compared with moderately defended prey (mass loss phase: $F_{1,92} = 15.57$, $P < 0.01$; mass gain phase: $F_{1,88} = 11.47$, $P = 0.001$) and also defended prey of both types were eaten for longer in a session as body mass decreased (mass loss phase: $F_{1,92} = 6.17$, $P < 0.05$; mass gain phase: $F_{1,88} = 13.41$, $P < 0.001$).

We also tested whether decisions to eat defended prey within a session could be influenced either by the amount of toxin or nutrients already eaten in a session. For each defended mealworm that was eaten, we measured the number of mealworms (undefended and defended) that had already been eaten in a session as a measure of energetic state and also the number of toxin units (mildly defended prey = 1, moderately defended prey = 3) that had been eaten prior to this decision. We then calculated the mean scores for each session and correlated these scores with the mean position in the session when both defended prey types were eaten. We did this for each phase separately. The mean position in a session was correlated with the total amount of mealworms already eaten in a session (mass loss phase: mildly defended prey $r^2 = 0.65$, $P < 0.001$ and moderately defended prey $r^2 = 0.97$, $P < 0.001$; mass gain phase: mildly defended prey $r^2 = 0.84$, $P < 0.001$ and moderately defended prey $r^2 = 0.86$, $P < 0.001$), and the toxin burden when the decision to eat was made (mass loss phase: mildly defended prey $r^2 = 0.25$, $P = 0.060$ and moderately defended prey $r^2 = 0.75$, $P < 0.001$; mass loss phase: mildly defended prey $r^2 = 0.61$, $P < 0.001$ and moderately defended prey $r^2 = 0.93$, $P < 0.001$). Therefore, decisions could have been made in response to changes in either of these physiological factors.

Latencies to attack

We tested whether the birds' nutritional states could also affect their latency to attack the 3 different prey types. In the mass loss phase, birds were quicker to attack prey in the last 2 days compared with the first 2 days ($F_{1,5} = 38.72$, $P = 0.002$). There was

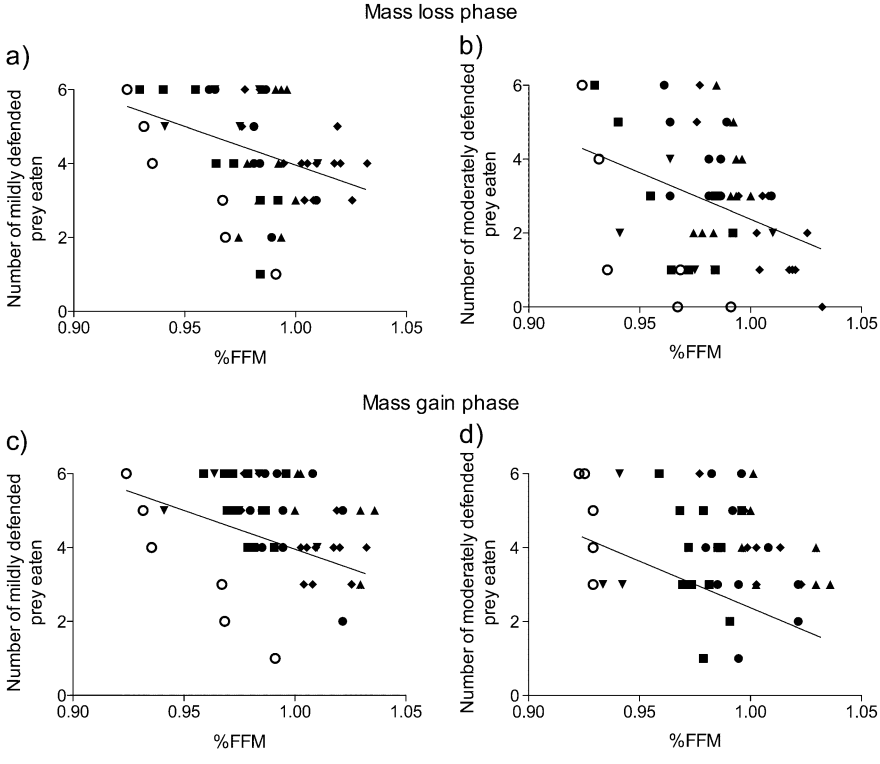

Figure 2
Decline in the numbers of mildly and moderately defended prey eaten in a session as the percentage FFM increased in the mass loss and the mass gain phases. Regression lines are shown for the untransformed pooled data. Symbols differentiate the data for each individual bird (see legend of Figure 1).

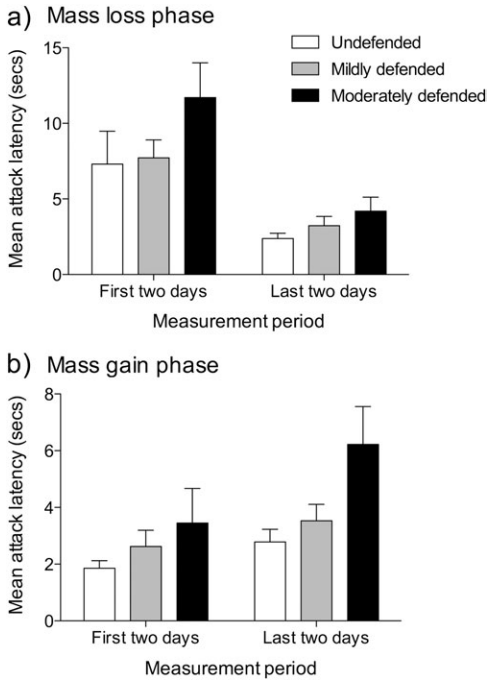

Figure 3
Mean (+standard error) attack latencies (seconds) for each prey type during the first 2 and last 2 sessions of (a) the mass loss phase and (b) the mass gain phase.

also a significant effect of prey type ($F_{2,10} = 4.15$, $P = 0.049$) but no interaction ($F_{2,10} = 0.76$, $P = 0.49$; see Figure 3a). In the mass gain phase, Mauchley's test indicated that the assumption of sphericity had been violated ($\chi^2 = 11.68$, $P < 0.01$), and therefore, dfs were corrected using Greenhouse–Geisser estimates of sphericity. In this phase, birds were not any quicker to attack prey in the first 2 days compared with the last 2 days of the phase ($F_{1,5} = 4.537$, $P = 0.086$; see Figure 3a), although the trend was in the expected direction. We also found a significant effect of prey type in the mass loss phase ($F_{1.03,5.24} = 9.17$, $P = 0.028$) but no significant interaction between the main effects ($F_{1.09,5.47} = 0.207$, $P > 0.5$). Therefore, birds took longer to attack prey with increasing amounts of quinine and also appeared to attack prey more quickly when they were in poorer nutritional state, at least in the mass loss phase.

Simultaneous choices

In the simultaneous choice sessions, birds appeared to discriminate between mealworms on the basis of color and attacked at random when color cues were absent (see Table 2). In both the Color–quinine and the Color-only treatments, birds showed preferences for the least defended prey type in each pairing, although the preference for mildly over moderately defended prey was not significant in the Color-only condition, perhaps because birds were able to relearn that the prey were no longer toxic. In the Quinine-only treatment where there were no color cues present, birds showed no preferences for the least defended prey types.

DISCUSSION

Our results show that birds can learn to use color signals to regulate their intake of variably toxic prey according to their current energetic state. In line with previous studies, we have found that decreasing energetic state leads to an increase in ingestion of prey containing toxins (e.g., Sexton et al. 1966; Gelparin 1968; Hileman et al. 1995; Barnett et al. 2007). However, in contrast to earlier work, this study takes an important step by showing that educated predators make strategic decisions to ingest less toxic prey compared with more toxic prey when they are visually distinct and that this preference continues to exist across changes in energetic state. In addition, our study shows that birds are more hesitant to attack defended compared with undefended prey. These findings are relevant for our understanding of the evolutionary dynamics of aposematism and mimicry, but more broadly, they provide insight into the cognitive mechanisms underlying nutrient–toxin trade-offs in animals.

Our data suggest that conspicuous coloration would be more costly to mildly defended prey compared with moderately defended prey when being hunted by educated predators. If educated predators in the wild learn about the toxicity of prey and are more likely to incorporate mildly compared with moderately defended prey into their diets, mildly defended prey should be selected to reduce the ease with which they are found. Therefore, we might expect a positive correlation between conspicuousness and toxicity (see also Blount et al. 2009), which has been found in some signaling systems (Summers and Clough 2001; Bezzerides et al. 2007; but see Darst et al. 2006). We might also expect that mildly defended prey would be selected to be conspicuously colored to enhance avoidance learning but be cryptic at a distance to reduce the probability of detection (Marshall 2000). Clearly, we need to know whether this could be the result of educated

Table 2
Mean (±standard error) number of each prey type eaten during the 9 simultaneous choice trials

Simultaneous choice test	Undefended	Mildly defended	Moderately defended	*t*	*P*
(a) Quinine–color					
Undefended versus mildly defended	14.5 ± 0.96	2.5 ± 0.89		6.78	<0.001
Undefended versus moderately defended	13.0 ± 1.15		1.67 ± 0.61	7.68	<0.001
Mildly defended versus moderately defended		9.0 ± 1.98	2.0 ± 0.58	3.53	<0.05
(b) Color-only					
Undefended versus mildly defended	13.83 ± 0.79	3.17 ± 0.54		8.32	<0.001
Undefended versus moderately defended	14.0 ± 0.68		2.33 ± 0.92	7.79	<0.001
Mildly defended versus moderately defended		7.83 ± 1.30	4.17 ± 1.17	1.71	ns
(c) Quinine-only					
Undefended versus mildly defended	7.33 ± 1.23	6.50 ± 1.02		0.65	ns
Undefended versus moderately defended	6.33 ± 1.27	5.33 ± 0.61		0.70	ns
Mildly defended versus moderately defended		6.00 ± 0.58	7.33 ± 0.80	2.39	ns

Statistics are the results from paired *t*-tests on each pairing. ns, not significant.

predators selectively eating less defended prey, and future experiments could manipulate toxicity and conspicuous in order to test this idea in the wild.

It is also intriguing to consider the role of attack latency in the evolution of aposematic signals. Latency has been widely used to measure naïve predators' responses to prey that are novel in appearance or carry typical warningly signals (e.g., Roper and Redston 1987; Skelhorn and Ruxton 2006; Marples et al. 2007) and also sometimes as a measure of avoidance learning (e.g., Roper 1993). However, to our knowledge, these are the first data from educated birds showing how attack latency can vary with prey toxicity and predator state. If predators are more hesitant to attack defended prey in the wild, it could allow defended prey time to escape and explain the evolution of behavioral escape strategies in aposematic prey. For example, buckmoth caterpillars (*Hemileuca lucina*) drop to the ground if disturbed by a potential predator (Cornell et al. 1987). This behavior in predators could also be extended to explain why slow movement is a trait commonly associated with toxicity and conspicuous coloration (e.g., Srygley and Chai 1990; Hatle et al. 2002; Sherratt, Rashed, et al. 2004). If highly defended prey have more time to escape predators, then selection for escape behavior might be relaxed on defended prey (Srygley and Chai 1990), leading to it becoming a reliable signal of toxicity (Sherratt, Rashed, et al. 2004). In addition, slower attack latencies on defended prey might promote better assessment of prey toxicity upon attack, which could stabilise the investment in defenses (Guilford 1994). All these selection pressures would vary with predator state, and the behavioral adaptations of aposematic prey might therefore depend upon what other more palatable prey were available.

Our study also has implications for recent state-dependent models of the evolutionary dynamics of mimicry (Kokko, et al. 2003; Sherratt 2003; Sherratt, Speed, et al. 2004). The diet choices and behavior of the birds supports the basic assumption of these models that educated predators can learn to recognize visually distinct prey that differ in their level of toxin and preferentially ingest more of the less toxic species. Although we have previously shown that birds can discriminate between, and make state-dependent foraging decisions on, variably defended prey (Skelhorn and Rowe 2010), this is the first study to show that they can do so on the basis of coloration. However, our findings do not support all the results from the model by Sherratt, Speed, et al. (2004). Curiously, this model predicted that visually distinct, mildly defended prey ("mimic controls" in Sherratt, Speed et al. 2004) would become acceptable to predators at higher energy levels as the predators' toxin burdens increased. This is in direct contrast to the behavior of birds in this experiment, with both defended prey types becoming less acceptable as toxin burden increased. This is expected because birds in a poorer energetic state should be more likely to value the nutrients that the toxic prey contain and more readily accept defended prey. It may therefore be necessary to change some of the parameters of the model to realistically capture the foraging behavior of predators in order to make more accurate predictions about the evolution of mimicry.

These data also have wider implications for how animals make decisions about eating toxic foods and how they regulate their toxin intake. Analysis of the birds' behavior within a session showed that they continued to eat the mildly defended prey for longer in a session compared with moderately defended prey. This suggests that birds not only had learned the relative toxicity of the 2 prey types but made strategic decisions about eating them according to the amount of nutrients and toxins they had consumed. As each session progressed, the energetic state of the birds improved as more mealworms were ingested, but toxin burden also increased as more defended prey were eaten. It is therefore difficult to know whether it was the increasing toxin burden or nutritional state (or an interaction of the 2) that reduced the attacks on defended prey. Although studies, particularly on plant–herbivore interactions, have provided widespread evidence for toxin–nutrient trade-offs (e.g., Simpson and Raubenheimer 2001; Villalba et al. 2002; Wright et al. 2003; Moore and Foley 2005), the physiological and cognitive mechanisms by which animals balance their intake of nutrients and toxins are not well understood (Torregrossa and Dearing 2009). To our knowledge, our study is the first to convincingly demonstrate the role of state-dependent decision making in the selection of foods that vary in their toxin content. Therefore, our results could potentially have significant implications for the selection and ingestion of toxic food across a wide range of ecological contexts. For example, the "detoxification limitation hypothesis" predicts that herbivores should eat toxic food until they reach a critical level at which point they should switch to a less toxic food (Marsh et al. 2006). However, our results suggest that rather than there being a single threshold for toxin ingestion, animals will use their current state, both energetic state and toxin burden, to decide when to pay the costs of eating toxic foods. Therefore, current theories of herbivory could incorporate state-dependent decision making to develop a broader framework for the study of the grazing behavior and the evolution of plant secondary metabolites.

In conclusion, our study has demonstrated that a state-dependent approach to the study of the ingestion of toxic food can provide novel insights into the evolution of aposematic signals. Our results support the assumptions, but also challenge the outcomes, of recent state-dependent models of mimicry; and highlight the need to fully understand how educated predators trade off the ingestion of nutrients and toxins, in order to produce more realistic models for the evolution of aposematism and mimicry. Our study also has broader implications for other areas where nutrient–toxin trade-offs are studied, and in particular may provide the basis for a more general framework in which to study animals' decisions to forage on toxic food, and the evolution of defenses.

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