ORIGINAL PAPER

Can the dietary conservatism of predators compensate for positive frequency dependent selection against rare, conspicuous prey?

Nicola M. Marples · Johanna Mappes

Abstract   The evolution of conspicuous colouration in prey is puzzling because such coloration attracts the attention of predators. Anti-apostatic selection, in which rare prey forms are predated disproportionately often, is a second potential obstacle to the evolution of conspicuous colouration in prey, as bright novel prey forms are likely to be very rare when they first appear in populations. It has recently been postulated that dietary conservatism in predators, an extended feeding avoidance of novel prey, would allow novel conspicuous prey to survive and multiply despite anti-apostatic and conspicuousness effects. We tested this hypothesis for a novel prey type arising in an otherwise cryptic population, providing a direct test of whether anti-apostatic selection or the predators' wariness to attack the novel prey type is the more important force acting on the novel conspicuous prey. We conducted our experiment in the "Novel World"; an experimental system designed to test predators' foraging decisions in a large landscape. We found that the conspicuous, novel prey suffered high initial costs of conspicuousness compared with cryptic prey, since most of these prey were attacked during the first "generation", with no opportunity to "reproduce". However, a subset of the 17 birds (24%) were following a dietary conservative foraging strategy and they were reluctant to eat the novel prey. Interestingly these birds were not more neophobic or less explorative. Our data demonstrate how difficult it is for the novel conspicuous prey to survive in cryptic populations, but they also highlight the importance of the predator's foraging strategies in helping to promote the evolution and maintenance of aposematism.

Keywords   Frequency dependent selection · Dietary conservatism · Evolution · Aposematism · Bird

N. M. Marples (✉)
School of Natural Sciences, Trinity College, Dublin 2, Ireland
e-mail: nmarples@tcd.ie

J. Mappes
Centre of Excellence in Evolutionary Research,
University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland
e-mail: johanna.mappes@jyu.fi

Introduction

In the natural world, many species co-exist in several colour morphs, such that they can be easily distinguished by predators. For example the 10 spot ladybird *Adalia decempunctata* L. has three common conspicuous colour morphs, peppered moth *Biston betularia* L. has two cryptic morphs, and garter snakes *Thamnophis elegans* Baird and Girard have many morphs, some of which are cryptic and some conspicuous. How such polymorphisms can be maintained in natural populations has piqued the curiosity of evolutionary biologists over several decades (review in Gray and McKinnon 2007).

Assuming that predation is the main selection factor influencing prey colouration, it has been suggested that negative frequency dependent selection (also called apostatic selection) can maintain such colour polymorphisms (e.g. Greenwood 1984; see review Sinervo and Calsbeek 2006). Predators are often more successful at detecting and consuming common cryptic prey morphs once they are familiar with that morph; a phenomenon known as "search image" (e.g. Tinbergen 1960; Ford 1975; Pietrewicza and Kamil 1981; Allen 1988). A predator using a search image therefore favours rare morph survival, even if that morph is slightly more conspicuous than the common morph(s). This results in negative frequency dependent selection (Clarke 1962; Allen 1988) and provides one mechanism by which polymorphisms may persist (Bond and Kamil 1998; Bond and Kamil 2002).

However, if a mutation gives rise to a new morph which is much more conspicuous than the rest of the parent population, then no learning is required to find it, and the formation of a search image is unnecessary. Under these circumstances, two processes work together, both tending to reduce the survival of the new morph. Firstly, the enhanced conspicuousness of the new prey morph attracts the predator's attention to it. Secondly, positive frequency dependent selection (also known as anti-apostatic selection) is expected to occur, in which the predator attacks the rarer form of the prey i.e. the new morph, more frequently than expected. Thus predation selects against the survival of the new, rare, conspicuous form. Such positive frequency dependent selection has been argued theoretically (Clarke 1962; Allen 1988; Endler 1988) and demonstrated empirically (e.g. Church et al. 1997; Lindström et al. 2001a; Allen and Weale 2005).

Despite such predation pressures, some prey are brightly coloured and very conspicuous, which is usually a signal of toxicity or some other unprofitability. Such advertising is called aposematism (Poulton 1890). The evolution and survival of aposematic species is a paradox because positive frequency dependent selection would be expected to cause the extinction of the rare new conspicuous prey before the predators have learned to avoid it. Thus, aposematism can only be beneficial when the population size of aposematic prey reaches some threshold abundance (Fisher 1930; Endler 1988; Lindström et al. 2001a), after which, the cost of teaching the predators the meaning of the signal is outweighed by the benefit of avoidance by educated predators (e.g. Gittleman and Harvey 1980; Guilford 1990; Alatalo and Mappes 1996; Lindström et al. 1999a).

Many solutions to the paradox of the origins of aposematism have been postulated (see recent review in Ruxton et al. 2004). One of the most recent, dietary conservatism (DC), is evaluated in the present study and described below. Marples et al. (1998) observed that many predators are not prepared to attack prey simply according to their visibility, but clearly prefer familiar prey, avoiding any novel form of that prey, even if it is more conspicuous. Such wariness in attacking new items consists of two processes; neophobia (Brigham and Sibley 1999) which is a fear of contact with the item, and which lasts only for a matter of minutes in birds, and dietary conservatism (Marples et al. 1998), which is a

relatively long lasting refusal of some individual predators to accept novel prey morphs as part of their diet (persisting for days to months). Dietary conservatism has been shown to have a genetic basis in Japanese quail *Coturnix japonica* Temminck and Schlegel (Marples and Brakefield 1995). All populations of bird predators tested so far (in 7 species: European robin *Erithacus rubecula* L., European blackbird *Turdus merula* L., zebra finches *Taeniopygia guttata* Vieillot, blue tit *Cyanistes caeruleus* L., Japanese quail, Asian blue quail *Excalfactoria chinensis* L., and domestic chicken *Gallus gallus domesticus* L.) have some individuals (30–50% of the population) which show DC, while the rest of the population attack novel prey types as soon as their neophobia has waned (Marples and Kelly 2001; Marples et al. 2005). These foragers are considered to be "adventurous consumers" (AC) (Thomas et al. 2010). The presence of DC foragers in most populations has been suggested as a mechanism which would allow rare conspicuous morphs to become common in some predator territories, and so achieve population levels high enough to allow aposematism to evolve despite the cost of educating the predators (Thomas et al. 2003, 2004).

In previous experiments on the effects of DC predators (Marples et al. 1998; Thomas et al. 2003, 2004) the experimental design did not accurately reflect the foraging task for birds in the wild, in some important ways. For example, the "cryptic" parent population of prey were simply the same colour as the background and three dimensional, so not really difficult to find. Furthermore the prey were presented in a small tray, so the birds probably spent very little extra effort searching for them. The prey population was also sufficiently small (20 prey items) to allow the novel morph to reach fixation very quickly (<10 "generations" i.e. trials). Furthermore, the previous experiments presented the choice between novel and familiar prey simultaneously, which would have the effect that any predator biases would be more easily expressed than in a sequential prey choice. It is unclear which is the more common natural situation, but sequential prey choice is at least often the case in the wild. It is therefore important to test the occurrence of DC under more stringent experimental conditions in order to give a more realistic estimate of its strength as a potential force to balance selection against conspicuousness.

In this experiment, we tested which predator behaviour is the more important in determining the survival of a rare conspicuous prey: positive frequency dependent (i.e. anti-apostatic) selection or the dietary conservatism of the predators (favouring the survival of the rare conspicuous form). We tested this question in the "Novel World" (an artificial landscape holding artificial prey; Alatalo and Mappes 1996; Ihalainen et al. 2007) in which it is easy to manipulate the relative conspicuousness of different prey morphs. An additional benefit of this method was that both cryptic and conspicuous prey were marked with black and white symbols not colours, and thus it was unlikely that any biases in the predators' responses to colours would have confounded their foraging decisions. In previous experiments, birds have not shown any biases for or against cross and square symbols (e.g. Alatalo and Mappes 1996; Lindström et al. 1999a) and neither was there any evidence that conspicuousness *per se* in such a situation would work as a signal of unpalatability (Sillén-Tullberg 1985; Lindström et al. 1999b). The prey population size which could be tested in the large arena available in "Novel World" was much larger and at a more realistic density than in the previous experiments, making the choice by the birds more like a sequential situation than a simultaneous situation.

In the present evolutionary experiment we investigated whether a rare conspicuous morph (1% of the prey population) could survive and spread in a truly cryptic population of prey under predation by a bird predator. To mimic the advent of a new aposeme in the wild, we modelled empirically the mutation of a novel conspicuous morph of a palatable

cryptic species, where the predators were familiar with consuming the cryptic prey prior to the introduction of the novel palatable morph into the prey population. Such a situation would occur if conspicuousness evolved before unprofitability in a newly aposematic species. In addition, the prey were not unpalatable so that any prey items which survived would do so because of aversion to novelty, not learned avoidance. In that sense the experiment represents the situation where conspicuousness evolved before a chemical defence.

Materials and methods

Seventeen wild great tits *Parus major* L. were trapped at a feeding station and ringed for identification. Each bird was kept individually in a plywood cage 65 cm × 50 cm × 80 cm and illuminated with two energy saving 7 W light bulbs. These provided a daily light period of 11.5 h. Sunflower seeds, peanuts and fresh water were available ad libitum except prior to the experimental trials. Birds were food deprived for between 1 and 2 h to ensure motivation to search for the artificial prey prior to each trial. The experiment was run from October to November 2006 at the Konnevesi Research Station in central Finland by permission from the Central Finland Regional Environment Center (permission numbers KSU-2006-L-249) and the Experimental Animal Committee of the University of Jyväskylä (permission numbers 35/22.5 2006). After the experiment, the birds were released at their site of capture. The data were analysed with SPSS 16 for Windows. All tests were two-tailed, and non-parametric tests were used where the data did not meet the requirements of parametric tests.

Artificial prey

The prey items were small pieces (approximately 0.1 g in weight) of almond glued with non-toxic glue (UHU Stick) between two 8 mm × 8 mm pieces of paper. One black-and-white signal was printed on both sides of the paper shell of the prey items. A cross symbol was also printed on the background on aviary floors creating distracters for the cryptic prey items (see Aviaries section). Familiar prey were marked with crosses, and the novel, conspicuous prey pattern was a black printed square.

Aviaries

The experiments were carried out in a large aviary which measured 3.5 meters high with a floor area of 57 m^2. The floor was covered with white, A3 sized paper sheets, which were glued together and covered with adhesive plastic. There were 70 printed crosses placed at a variety of angles and spacing, and ten fake cryptic prey items in random positions on each sheet. The fake prey items (8 mm × 8 mm pieces of cardboard with printed crosses glued on the top) were glued onto the sheets to make the background three-dimensional, in order to camouflage the cryptic prey better. The A3 paper sheets were placed on the floor to form a continuous grid of 15 rows and 22 columns. There were wooden dividers (ca. 6 cm wide) between each row, to facilitate prey handling and movement of the birds. There were eight perches at a height of 0.5 m to allow birds to perch while handling prey.

In the experimental setup, prey density was low, with a maximum of one prey item per paper sheet. This also enabled identification of the attacked items by their coordinates.

During the experiments, the birds were observed through a one-way mirrored window. Fresh water was always available in the aviary.

Training of the birds

All birds were trained to handle the artificial prey in their home cages in four steps by offering them (1) small almond slices (2) five-one-sided prey items, a piece of almond glued onto white pieces of paper with crosses printed on each, sized 8 mm × 8 mm (3) five prey items with the almond sticking out from the paper shell and finally (4) five prey items with the almond completely hidden inside the paper shell. The birds had to eat all items before their training progressed to the next phase.

Before the experiment, the birds were trained in small aviaries (2.4 m high with a floor area of 13.5 m^2) to forage for cryptic prey, which was marked with crosses against the background bearing cross symbols, as described above. The floors of the small aviaries were covered with eight rows of ten A3 paper sheets. Thus the birds were familiarised with finding the cryptic prey on the same type of background as in the experiment. The birds were sufficiently familiar with the experimental set up that they would readily search for familiar prey in the experiments. During the training in the small aviary, we placed 15 cryptic prey items on the floor so that they were in groups of three. One prey item in each group was placed on a divider, so that it was clearly visible. The second prey item was close to the board and the third was in the middle of the sheet. Each bird had to find and eat all prey items. The training ensured that the predators had enough experience of finding the cryptic prey to consider it the familiar prey type. To familiarise the birds with the large experimental aviary, all the birds were allowed to feed and spend the night in it as a group. During this period of familiarisation with the large aviary, the experimental background was replaced by transparent plastic with peanuts, mealworms and sunflower seeds placed on the floor, to encourage the birds to feed on the floor.

Experimental tests

The experimental aviary was set up with the cross symbol background and wooden dividers in place. This provided 330 A3 sheets each of which could be considered a foraging block, labelled by its coordinates in rows and columns. The four sheets under each of the eight perches located in the landscape and two sheets under each of the four wall attached perches were kept empty of prey. This was done to ensure that any dropped items could be distinguished from undiscovered prey. Thus there were 290 possible blocks into which a prey item could be placed. We placed 99 familiar, cryptic baits (marked with a cross) and one novel, conspicuous bait (marked with a black square) at randomly selected co-ordinates, so that only one bait was present in any one block. A different map of random bait positions was used for each trial of each bird.

We allowed each bird in turn to forage in the experimental aviary, and eat a maximum of 50 prey items, or for 2 h from their first prey item taken. The baits were considered to have been "killed" if they were torn open, and to have "survived" if the packet was left intact even if it was handled, or taken to a perch. At the end of this time, the number of each type of prey remaining was counted, and the population multiplied up to a total of 100 baits in proportion to the number of each bait type left. This formed the next "generation" of prey which that particular bird would meet in its next trial. The birds were given repeated trials, one per day, until one or other of the two prey types went extinct (following the methodology of Thomas et al. 2003).

Table 1 The proportion of prey populations with surviving novel prey after selected numbers of generations, expressed as a percentage of the total populations tested: 10,000 populations were tested for the simulations, 17 for the empirical experiment

Generations	% Populations; birds selecting by frequency	% Populations; birds selecting by visibility	Observed result
3	30.25	0.77	23.5
6	19.56	0.01	17.6
13	11.86	0	5.8
19	8.37	0	5.8

Simulating expected attack risk for novel prey

In order to interpret our empirical results, a measure of the expected survival of the novel prey was needed. To generate this, two simple simulations were run using the R statistical programme. First we asked how many attacks a predator would be expected to make on cryptic prey before it attacked a novel prey, if the predator selected its prey randomly and in proportion to the frequency of the two prey types (initially 99 cryptic versus 1 novel). In this simulation we took into account the changing morph frequencies during the course of the trial, so, for instance, the likelihood of attacking novel prey increased as cryptic prey were eaten.

In a second prediction using this simulation, we incorporated the greater conspicuousness of the novel prey. To do this, we took into account that the square marked baits have previously been shown to be approximately three times more conspicuous than the cross marked baits (Riipi et al. 2001; Ihalainen et al. 2007). Thus we ran the simulation as before, but this time gave the novel prey type a three times higher predation risk compared to the cryptic prey (Riipi et al. 2001; Ihalainen et al. 2007). Each simulation was repeated 10,000 times, and we recorded the number of cryptic baits which would be attacked before the first novel prey item was contacted in each population, and the number of cryptic baits attacked before the first novel prey was eaten.

A second simulation was used to estimate the length of time for which the novel prey type would persist in a population if it started at a ratio of 1 novel conspicuous: 99 cryptic familiar prey, and followed the "breeding rules" set out in the empirical study above. To this end, 50 prey items were consumed each trial ("generation"), and the population was multiplied in proportion to the number of each bait type left at the end of the generation. This was continued for a maximum of 19 generations in the simulation. Since we wanted to compare our empirical data to the simulated data, we specifically asked how likely it was for the novel prey population to survive three generations, six generations, 13 generations and 19 generations (see Fig. 2; Table 1) if birds attacked them according to relative frequencies of each prey type. Again we repeated the simulation allowing for the conspicuousness of the novel prey type by assuming a three times higher predation risk for the novel prey. Each simulation was run 10,000 times.

Results

Our first simulation predicted the expected number of cryptic baits which would be eaten before the first novel prey was attacked. When the predator was assumed to attack according to the frequency of the prey, this gave a prediction of 57.81 ($\pm$42.12) cryptic baits (Fig. 1 dotted line).
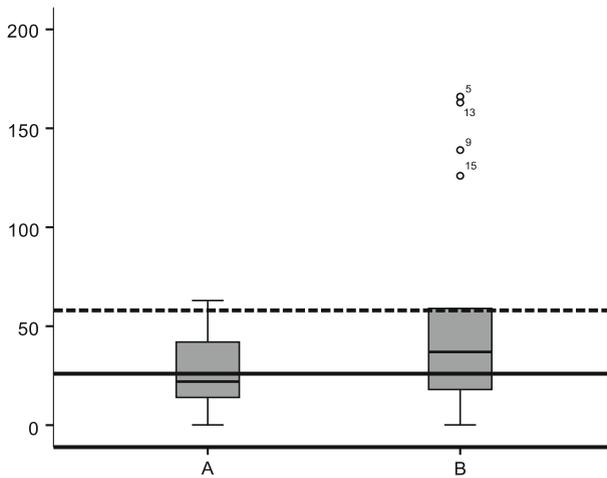
Fig. 1 Longevity of novel prey, calculated as the number of cryptic prey encounters before (*A*) the novel bait was contacted (pecked, handled, or eaten), and (*B*) the novel bait was eaten. The *dotted line* at $y = 58$ shows the expected longevity of the novel prey according to its frequency, calculated from simulated data (mean 57.8 ± 42.11 SD). The *solid line* at $y = 26$ represents the expected longevity of the novel prey according to the visibility, given that the novel, conspicuous prey was approximately three times as conspicuous as the familiar, cryptic prey (mean 25.69 ± 19.86 SD). *Box plots* show minimum and maximum, and the upper and lower quartiles around the median. The outliers are denoted by *open circles*

We used this estimate in two ways. In the first analysis we looked at how long it took the birds to make contact with the novel prey, and so we counted pecking at the prey, handling it or eating it as the end of its "lifespan". Under these circumstances (Fig. 1A) the birds contacted the novel, conspicuous prey earlier than would be expected if they had encountered prey at random (Welch two sample t-test between observed and simulated data $t = 7.76$, $df = 16.38$, $P < 0.001$).

In a second analysis we considered the novel prey to survive unless it was eaten, and so did not count handling by the birds. Under these circumstances the observed lifespan of the novel prey did not differ from the expected value obtained from the simulated data (Fig. 1B; $t = 0.12$, $df = 16.03$, $P = 0.909$).

In a further analysis, we incorporated the difference in the conspicuousness of the familiar and novel prey types, simulating the longevity of the novel conspicuous prey taking into account its difference in visibility compared to the cryptic prey. According to these simulations, the "death" of the novel prey should have occurred, on average, within the first 26 prey items "killed" (mean 25.69 ± 19.86) (Fig. 1 solid line).

As before, we first looked at how long it took the birds to encounter the prey, i.e. to make any contact, irrespective of whether they went on to eat it (Fig. 1A), and then how many cryptic, familiar prey were eaten before the birds ate the novel prey (Fig. 1B). We found that the novel prey were contacted at the rate expected from their conspicuousness (Welch two sample t-test between observed and simulated data $t = -0.47$, $df = 16.08$, $P = 0.642$). However, birds then went on and ate the novel prey significantly later than would be expected from their conspicuousness ($t = -2.25$, $df = 16.01$, $P = 0.0386$).

This experiment therefore showed that although the risk of rare conspicuous prey being contacted (handled) was high, it is notable that birds consumed the conspicuous prey later than would be expected from their visibility. This indicates that the decision whether to

handle novel prey (neophobia) was different from the decision to eat it (see Marples and Kelly 2001).

Survival of novel prey populations

In most of the empirically tested populations (13 out of 17 = 76.5%), the conspicuous novel bait went extinct within the first three trials (i.e. within the first 150 baits eaten), leaving 4/17 (23.5%) populations in which the novel prey survived three trials or more. We compared these empirical data to a simulation which estimated how often we would expect the novel prey to survive three generations in the same conditions as in our empirical experiment (Table 1). In the first version of this simulation the birds were assumed to eat the two prey types at random, according to their frequency, and under these conditions 3,025 populations out of 10,000 (30%) allowed the conspicuous prey to survive for three generations. Thus, our birds drove the novel prey to extinction more often than predicted. If, however, the conspicuousness of the novel prey was taken into account in the simulation, the simulation predicted far lower survival of the novel baits than was observed in the empirical tests (Table 1).

In four populations the real birds allowed the novel morph to persist for several trials, and even to increase in number to some extent (Fig. 2). In three of these populations, the novel prey eventually went extinct within 13 trials, but one bird (number 9) avoided novel prey for long enough to allow it to multiply and become the majority of the population. Thereafter, even though the bird stopped avoiding novel prey, and ate both prey types at random, neither reached extinction. Instead, the novel prey morph was maintained at between 50 and 60% of the population until the experiment was terminated, after 19 trials.
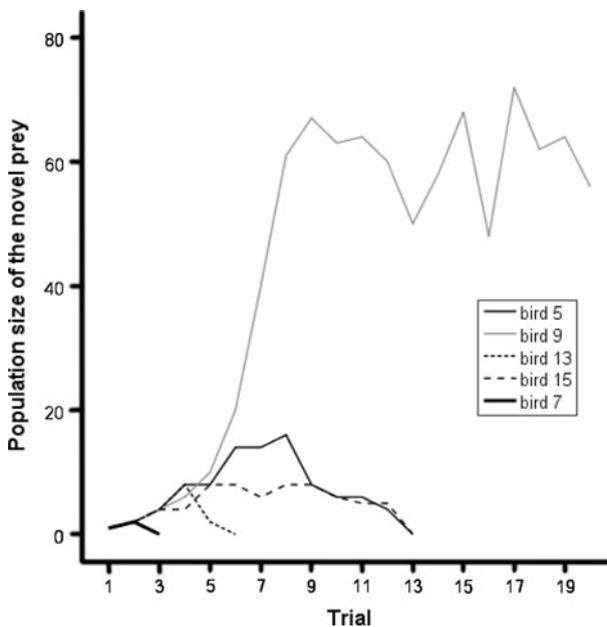

Fig. 2 The population size of novel prey at the start of each successive trial during the course of the experiment, plotted separately for all birds which allowed the novel prey to survive longer than the first trial

Fig. 3 The latency to contact the first prey item (novel or familiar) for each bird, plotted against the latency to eat the first novel prey item. *Open symbols* represent birds which allowed the prey to survive for more than two trials
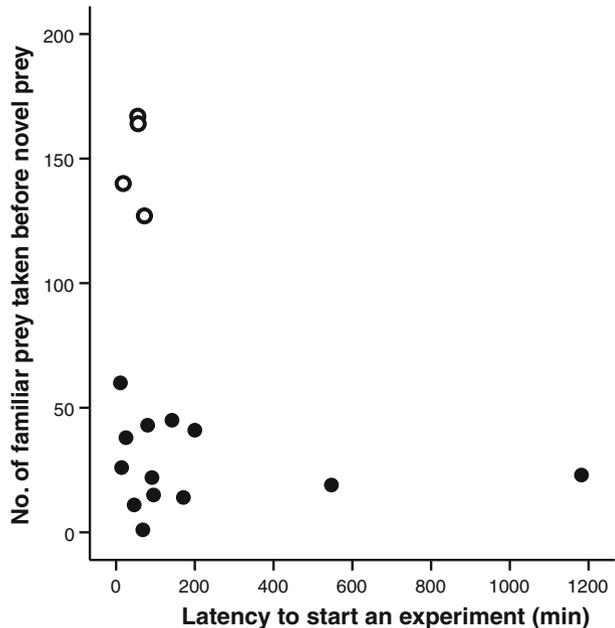
In the simulated data, only four out of 10,000 populations (0.04%) allowed the novel prey population to grow to 50 individuals and none allowed it to reach 60 individuals, as was observed in the empirical experiment.

When we took into account the higher conspicuousness of the novel prey, our simulations predicted very few populations out of the 10,000 which survived more than a few generations, and none that should survive more than 7 generations (Table 1).

Following the nomenclature of Marples et al. (1998) it is possible to consider the birds which avoided the novel prey for longer than the prey's expected longevity to be using a "Dietarily Conservative" foraging strategy (DC). The remaining birds, which ate the novel prey within the expected number of encounters, can be considered to be using an "Adventurous Consumer" foraging strategy (AC) (following Thomas et al. 2010).

One might argue that the suggested DC birds were showing the personality trait "exploration avoidance" and that AC birds were showing the opposite personality trait in which the birds are exploratory (Dingemanse et al. 2002; Reale et al. 2007). The exploration avoidance syndrome has been shown to be correlated with neophobia, both of food, and of new environments (Reale et al. 2007). We might therefore expect the DC birds to be slower to start foraging on arrival in the experimental room (c.f. open field tests), but there was no significant difference in the latency to start foraging between AC and DC birds (Mann–Whitney U-test: $U = 15.0$, $n_1 = 13$, $n_2 = 4$, $P = 0.21$). If anything, the DC birds seemed to start foraging faster than the mean for the AC bird group (Fig. 3).

Another intriguing difference in behaviour between the suggested AC and DC groups of birds, was the frequency with which they handled the prey of either morph but did not eat it. For instance, the birds would peck at, or sometimes pick up the prey and even fly to a perch with it, but then drop it without opening the bait. Such prey were said to have been "handled" (as opposed to eaten). We only considered the handling during the first trial in each case so that we could compare all the birds, as many of the AC birds only participated

in the experiment for a single trial, after which the novel prey had become extinct. All four DC birds handled the novel prey in their first trial between one and four times each (total 10 cases of handling). This handling occurred in DC birds significantly more often than in the AC birds, which never handled the novel prey (0 cases) without proceeding to eat it. This difference was statistically significant (Fisher's Exact test, $P = 0.002$). The cryptic familiar prey were also handled more by DC birds than by the AC birds ($U = 7.0$, $n_1 = 13$, $n_2 = 4$, $P = 0.03$), with 6.9% of encounters with familiar prey items leading to the prey being rejected by DC birds, while only 1.6% of encounters with familiar prey led to rejection by AC birds. This demonstrates that the DC birds were not actually afraid of the novel prey items (neophobic), contacting them regularly, but were generally less willing, or more cautious in consuming any prey (familiar as well as novel) than the AC birds. (See also the distribution of outliers in Fig. 1). This suggests that the AC:DC behavioural differences are distinct from neophobia, a reluctance to contact the prey, but are probably part of a foraging strategy, as argued by Marples and Kelly (2001). An alternative explanation, that the birds described as DC were simply less hungry than the AC birds is not supported by the data, as there is no evidence that they were slower to start eating (Fig. 2).

Discussion

This experiment aimed to compare the strengths of the effects of positive frequency dependent selection and dietary conservatism on the survival of a novel conspicuous prey item appearing in a population of cryptic prey. Positive frequency dependent selection has been suggested to be the major force preventing the spread of such morphs (Greenwood 1984; Endler 1988; Lindström et al. 2001a). The strength of this selection pressure has recently been called into question however, as some predators have been observed to be reluctant to accept the novel prey into their diet (Thomas et al. 2003, 2004; Marples et al. 1998), allowing the novel prey to spread. Our results suggest that frequency dependent selection is an important force affecting the survival of the rare novel morph, particularly if the prey is soft bodied, since the novel morph was contacted more often than would be expected from their frequency in the population. Moreover, most populations of novel prey went extinct within the first three trials, which is sooner than could be expected if birds selected their prey purely according to their frequencies (Table 1). This supports the existence of a paradox concerning the initial evolution and maintenance of conspicuous morphs (e.g. Fisher 1930; Endler 1988; Guilford 1990; Alatalo and Mappes 1996) because prey pay a cost to being attacked, even if they are not then eaten, which is likely to reduce their ability to reproduce. However, it is important to note that while birds attacked novel prey sooner than would be expected, they ate the novel prey later than their conspicuousness would suggest (Fig. 1). This result was due to the fact that there were clear outliers that delayed consuming prey much longer than could be expected from either the novel prey availability or visibility. This suggests that some birds were wary of consuming novel conspicuous prey and this avoidance resulted in a higher than expected survival for the conspicuous prey encountered by these individuals. The results suggest that the birds may have been showing two distinct sets of behaviour, some birds showing extended wariness towards eating novel conspicuous prey (dietary conservatism, DC) while others accepted the novel prey readily (adventurous consumption, AC) (see Fig. 1). Lindström et al. (1999a) and Riipi et al. (2001) also found that novel, conspicuous prey suffered a cost of conspicuousness, but the prey were attacked less than might be expected according to their

visibility risks, although the variation in predator behaviour was not analysed. A further experiment by Lindström et al. (2001b) demonstrated that conspicuous prey did not suffer any costs of conspicuousness if cryptic prey were familiar to the predators, suggesting that search image formation allowed the birds to find the cryptic prey more easily. However, in their experiment, the frequency of novel conspicuous prey was high (50%) and thus anti-apostatic effects would have been difficult to detect.

It has been suggested that the behavioural syndrome "exploration avoidance" correlates with neophobia towards food, novel objects and novel environments (Reale et al. 2007). However, neophobia (willingness to *contact* novel objects) and dietary conservatism (reluctance to *eat* novel food) do not necessarily correlate (Marples and Brakefield 1995; Kelly and Marples 2004; Siddall and Marples 2008). In the present experiment, all four of the wary birds handled the novel prey regularly, and showed no hesitation in contacting it. Thus dietary conservatism appears to be different from neophobia. We therefore would not necessarily expect a correlation between dietary conservatism and "exploration avoidance", as measured by the willingness to enter a new space, and indeed, we found no such correlation. The wary birds were, if anything, faster to begin their foraging in the novel room.

Variation in predator foraging behaviour can have evolutionary consequences for both predator and prey. It may be important not simply to focus on the average predation effect on the prey population as if predators were homogeneous, but to consider the variation among predators in their responses to the prey populations. For example if the predators' foraging territories do not overlap or they overlap only partially, it is possible that a conspicuous mutant would survive in the territory of a conservative forager for a sufficiently extended period of time to allow it to reproduce, and perhaps disperse to other territories (Marples et al. 2005). This might therefore assist the evolution of aposematism or the maintenance of unprofitable conspicuous novel prey types, especially for tough bodied insects which can survive investigative handling (Wiklund and Järvi 1982). It would also give a palatable novel prey type longer in which to evolve unprofitability, so opening an alternative path to aposematism (Thomas et al. 2003, 2004; Marples et al. 2005) although this route to aposematism has been suggested to be much less likely than other routes (Guilford 1990; Sherratt 2002).

In our experiment all prey were palatable, making the spread of the novel conspicuous prey less likely as no avoidance learning could take place. Even so, one population in our experiment survived 19 generations and the frequency of the novel prey increased to more than 60% of the population. Our simulations suggest that this is very unlikely to happen unless predators are actively avoiding this prey type, since only 0.04% of simulated populations grew to 50 novel prey and none to 60 individuals in the population of 100 prey items. It has been shown that if a bird experiences unprofitability, then that unpleasant experience increases its level of wariness (Skelhorn and Rowe 2006a, b) making it even more reluctant to attack novel prey in the future (Marples et al. 2007). Foraging wild birds may therefore be in a state of heightened wariness most of the time, since they are likely to encounter aversive prey relatively often. It is thus probable that any novel conspicuous prey arising in a territory of the conservative bird would not only initially be allowed to spread, but may continue to do so as the bird continually reverts to novel prey avoidance. Before we can foresee the evolutionary consequences of predator foraging strategies it is necessary to know more about the frequency and duration of dietary conservative strategies in the wild, and their overall effect on prey populations, given that they are probably usually present only in an minority of the territories.

References

Alatalo RV, Mappes J (1996) Tracking the evolution of warning signals. Nature 383:708–710

Allen JA (1988) Frequency-dependent selection by predators. Phil Trans Roy Soc B 319:485–503

Allen JA, Weale ME (2005) Anti-apostatic selection by wild birds on quasi-natural morphs of the land snail *Cepaea hortensis*: a generalised linear mixed models approach. Oikos 108:335–343

Bond AB, Kamil AC (1998) Apostatic selection by blue jays produces balanced polymorphism in virtual prey. Nature 395:594–596

Bond AB, Kamil AC (2002) Visual predators select for crypt city and polymorphism in virtual prey. Nature 415:609–614

Brigham AJ, Sibley RM (1999) A review of the phenomenon of neophobia. In: Cowand DP, Feare CJ (eds) Advances in vertebrate pest management. Filander Verlag, Furth, pp 67–84

Church SC, Jowers M, Allen JA (1997) Does prey dispersion affect frequency-dependent predation by wild birds? Oecologia 111:292–296

Clarke BC (1962) Balanced polymorphism and the diversity of sympatric species. In: Nichols D (ed) Taxonomy and geography. Systematics Association publication no. 4. Systematics Association, Oxford, pp 47–70

Dingemanse NJ, Both C, Drent PJ, van Oers K, van Noordwijk AJ (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. Anim Behav 64:929–938

Endler JA (1988) Frequency-dependent predation, crypsis and aposematic coloration. Phil Trans R Soc Lond B 319:505–523

Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford

Ford EB (1975) Ecological genetics, 4th edn. Chapman & Hall, London

Gittleman JL, Harvey PH (1980) Why are distasteful prey not cryptic? Nature 286:149–150

Gray SM, McKinnon JS (2007) Linking colour polymorphism maintenance and speciation. TREE 22:71–79

Greenwood JJD (1984) The functional basis of frequency-dependent food selection. Biol J Linn Soc 23:177–199

Guilford T (1990) Evolutionary pathways to aposematism. Acta Ecol 11:835–841

Ihalainen E, Lindstrom L, Mappes J (2007) Investigating Mullerian mimicry: predator learning and variation in prey defences. J Evol Biol 20(2):780–791

Kelly DJ, Marples NM (2004) The effects of novel odour and colour cues on food acceptance by the zebra finch (Taeniopygia guttata). Anim Behav 68:1049–1054

Lindström L, Alatalo RV, Mappes J, Riipi M, Vertainen L (1999a) Can aposematic signals evolve by gradual changes? Nature 397:249–251

Lindström L, Alatalo RV, Mappes J (1999b) Inherited avoidance towards colour, gregariousness and conspicuousness—experiments with naive and experienced predators. Beh Ecol 10:317–322

Lindström L, Alatalo RV, Lyytinen A, Mappes J (2001a) Strong antiapostatic selection against novel rare aposematic prey. PNAS 98:9181–9184

Lindström L, Alatalo RV, Lyytinen A, Mappes J (2001b) Predator experience on cryptic prey affects the survival of conspicuous aposematic prey. Proc Roy Soc Lond B 268:1925–1929

Marples NM, Brakefield PM (1995) Genetic variation for the rate of recruitment of novel insect prey into the diet of a bird. Bio J Linn Soc 55:17–27

Marples NM, Kelly DJ (2001) Neophobia and dietary conservatism: two distinct processes? Evol Ecol 13:641–653

Marples NM, Roper TJ, Harper DGC (1998) Responses of wild birds to novel prey: evidence of dietary conservatism. Oikos 83:161–165

Marples NM, Kelly DJ, Thomas RJ (2005) Perspective: the evolution of warning coloration is not paradoxical. Evolution 59:933–940

Marples NM, Quinlan M, Thomas RJ, Kelly DJ (2007) Deactivation of dietary wariness through experience of novel food. Beh Ecol 18:803–810

Pietrewicza AT, Kamil AG (1981) Search images and the detection of cryptic prey: an operant approach. In: Kamil AG, Sargent TD (eds) Foraging behavior: ethological and psychological approaches. Garland STPM Press, New York, pp 311–332

Poulton EB (1890) The colours of animals. Their meaning and use. Especially considered in the case of insects. Kegan Paul, Trench, Trübner and Co. Ltd, London

Reale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318

Riipi M, Alatalo RV, Lindström L, Mappes J (2001) Multiple benefits cover detectability costs in aposematic aggregations. Nature 413:512–514

Ruxton GD, Sherratt TN, Speed MP (2004) Avoiding attack. Evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press, Oxford, p 249

Sherratt TN (2002) The coevolution of warning signals. Proc Biol Sci 269(1492):741–746

Siddall E, Marples NM (2008) Better to be bimodal: the interaction of colour and odour on learning and memory. Behav Ecol 19:425–432

Sillén-Tullberg B (1985) The significance of coloration *per se*, independent of background, for predator avoidance of aposematic prey. Anim Behav 33:1382–1384

Sinervo B, Calsbeek R (2006) The physiological, behavioural, and genetical causes and consequences of frequency dependent selection in the wild. Ann Rev Ecol Evol Syst 37:581–610

Skelhorn J, Rowe C (2006a) Taste-rejection by predators and the evolution of unpalatability in prey. Behav Ecol Sociobiol 60:550–555

Skelhorn J, Rowe C (2006b) Avian predator's taste-reject aposematic prey on the basis of their chemical defence. Biol Lett 2:348–350

Thomas RJ, Marples NM, Cuthill IC, Takahashi M, Gibson EA (2003) Dietary conservatism may facilitate the initial evolution of aposematism. Oikos 101:458–466

Thomas RJ, Bartlett L, Marples NM, Kelly DJ, Cuthill IC (2004) Prey selection by wild birds can allow novel and conspicuous colour morphs to spread in prey populations. Oikos 106:285–294

Thomas RJ, King TA, Forshaw HE, Marples NM, Speed MP, Cable J (2010) The response of fish to novel prey: evidence that dietary conservatism is not restricted to birds. Behav Ecol 21:669–675

Tinbergen N (1960) The natural control of insects in pine woods: Vol. I. Factors influencing the intensity of predation by songbirds. Archives Neelandaises de Zoologie 13:265–343

Wiklund C, Järvi T (1982) Survival of distasteful insects after being attacked by naïve birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. Evolution 36:998–1002