

# Butterfly effects in mimicry? Combining signal and taste can twist the relationship of Müllerian co-mimics

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**Abstract** Müllerian co-mimics are aposematic species that resemble each other; sharing a warning signal is thought to be mutually beneficial for the co-mimics by reducing per capita predation risk. In Batesian mimicry, edible mimics avoid predation by resembling an aposematic model species. The protection of both the model and the mimic is weakened when the mimics are abundant compared to the models. The quasi-Batesian view suggests that defended (Müllerian) co-mimics, when unequal in their defences, could also show a Batesian-like trend of increasing mortality with increasing abundance of a less defended “mimic”. We manipulated frequencies of unequally distasteful artificial co-mimics that were prey for great tits. The co-mimics had different signals (imperfect mimicry) but were equally preferred by the birds when palatable. Unexpectedly, when unpalatable, one of the signals was easier for the birds to learn to avoid. Consequently, during predator learning, the signal design of the prey strongly affected mortality of the co-mimics; there was an interaction between the signal and frequency treatments, but increasing the frequency of a less defended “mimic” did not increase co-mimic mortalities as predicted. In contrast, in a memory test that followed, the effect of signal design disappeared; if the birds had experienced high frequency of “mimics” during learning, co-mimic mortalities did subsequently increase. Since the effect of co-mimic frequencies on mortalities changed depending on the signal design of

the prey and predator experience, the results suggest that mimetic relationship may be an unpredictable interplay of several factors in addition to taste and abundance.

**Keywords** Aposematism · Quasi-Batesian mimicry · Predator psychology · Avoidance learning · Memory

## Introduction

Defensive mimicry, where two or more prey species resemble each other, is an antipredator strategy based on aposematism. Aposematic species are unprofitable as prey for example due to toxicity. Further, they communicate this unprofitability to predators via warning signals like conspicuous coloration (Poulton 1890). Two types of defensive (or protective) mimicry, Batesian and Müllerian, have traditionally been recognised.

In Batesian mimicry, an edible mimic species gains protection by resembling an aposematic model species i.e. by using the same warning signal (Bates 1862). Predators that have learned to associate the warning signal with the unpalatability of the model will also reject the edible mimics by sight (Brower 1958; Brower et al. 1960; Boyden 1976); the mimics exploit the defence of the models. Qualitatively, Batesian mimics should occur sympatric to their models (Bates 1862; Pfenning et al. 2001) and they should be rare compared to their models (Bates 1862; Fisher 1958; Sheppard 1959; but see Brower 1960; Nonacs 1985); both the mimics and their models are expected to have lowest per capita mortality when the mimics are rare (Lindström et al. 1997) because the frequent occurrence of edible mimics could hamper avoidance learning or otherwise encourage predators to attack all individuals with the same signal.

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The original theory of Müllerian mimicry (Müller 1879) assumes that predators kill a certain number of individuals with a certain signal before they learn to avoid it. When the predators have to learn to avoid only one signal instead of two (or several), it decreases an individual's risk of being killed. By sharing the warning signal, aposematic co-mimic species can benefit from the shared mortality costs of educating the predators to avoid that signal. Thus, in Müllerian mimicry the co-mimic species' relationship is thought to be mutualistic; an increase in the number of any of the co-mimics should benefit all, as the more individuals there are sharing the signal, the lower the per capita predation risk (Müller 1879). Consequently, rare signal variants should be at a disadvantage (see Mallet and Barton 1989; Joron and Mallet 1998; Mallet 1999; Kapan 2001).

Thus, one thing that separates Batesian and Müllerian mimicry is the edibility or inedibility of the mimetic counterparts which is hypothesised to cause very different evolutionary dynamics in the two systems (see e.g. Turner 1987; Huheey 1988; Joron and Mallet 1998; and also Lindström et al. 2004). It has long been acknowledged (see e.g. Marshall 1908) that variation in the secondary defences of Müllerian co-mimics could complicate this division of mimicry to Müllerian and Batesian. Bird predators can differentiate levels of chemical defence (Alcock 1970) and prey palatability/unpalatability is indeed often measured as acceptability to predators.

As differences in unpalatability are common within (e.g. Brower et al. 1968; Brower et al. 1972; Eggenberger and Rowell-Rahier 1992) and between prey species (e.g. Brower et al. 1963; Bowers and Farley 1990; Sargent 1995), predators may not find aposematic co-mimic species equally aversive. How this variation in unpalatability might or might not create mimetic relationships different to the mutualistic one proposed by Müller (1879) has been studied using mathematical models (Huheey 1976; Owen and Owen 1984; Turner et al. 1984; Speed 1993; Turner and Speed 1996; Mallet 1999; Speed 1999; Speed and Turner 1999; Kokko et al. 2003; Sherratt et al. 2004). If moderate unpalatability is less efficient in educating and deterring predators, a highly unpalatable co-mimic could suffer more attacks per capita in the presence of a weakly defended look-alike than when alone. A moderately defended co-mimic could then act as a parasitic, Batesian-like mimic (quasi-Batesian mimicry, see e.g. Speed 1993, 1999).

In experimental mimicry studies that use artificial prey (Speed et al. 2000; Skelhorn and Rowe 2004; Lindström et al. 2006; Skelhorn and Rowe 2006a; Ihalainen et al. 2007), variation in unpalatability has sometimes been disadvantageous to the mimetic prey and sometimes not. Contrary to the Müllerian assumption, bird predators have also learned to avoid relatively dissimilar novel co-mimics as well as

visually accurate co-mimics (Rowe et al. 2004; Ihalainen et al. 2007; but see Beatty et al. 2004). Overall, studies that focus on predator learning in Müllerian mimicry do not show a consistent trend for the significance of variation in unpalatability, signal or both in the co-mimic population.

In this study, bird predators (great tits *Parus major*) were learning to avoid artificial co-mimics that were presented in different frequencies. The co-mimics differed in unpalatability and were also visually imperfect co-mimics of each other. We chose to study visually imperfect co-mimics, as it is often assumed (if not well documented) that imperfect mimicry is common in nature (see e.g. review in Gilbert 2005). The moderately defended co-mimics (henceforth "mimics") were thus potentially recognisable to the birds (cf. Lindström et al. 2006; Ihalainen et al. 2007) as were their highly unpalatable counterparts (henceforth "models"). However, birds can generalise their avoidance between prey with inaccurate signals (e.g. models and edible mimics in Lindström et al. (1997); equally unpalatable co-mimics in Rowe et al. (2004)). In addition to the models and mimics, the birds were also offered visually distinguishable palatable cryptic prey that kept them motivated to search for food. The learning trial was also paired with a subsequent "memory test." We used the "novel world method" (Alatalo and Mappes 1996); an artificial black-and-white world where artificial prey items had black-and-white symbols that signalled their unpalatability, and alternative palatable prey had symbols which made them cryptic against the background used. The total number of co-mimics was kept constant in the treatments and the mimic was either very rare and the model common or both co-mimics were equally common. Increasing the frequency of the mimics increased variation and decreased the overall unpalatability in the co-mimic "population": we tested whether this degrades predator learning. We also studied co-mimic mortalities in more detail first hypothesising that mortalities of both co-mimics could increase with increasing mimic frequency, as in Lindström et al. (1997), where the ratio of aposematic models and visually imperfect edible (Batesian) mimics was manipulated. However, the detailed analysis revealed a more complex interaction between unpalatability and the actual signal design of the prey.

## Materials and methods

We carried out experiments from September to December 2001 and from September to October 2003 at Konnevesi Research Station in Central Finland. Great tits were used as predators of artificial prey items in the experiments. We had permission to keep wild great tits in captivity and use them in the study from the Central Finland Regional Environ-

ment Center (permission numbers 0901L0448/254 and KSU-2003-L-396/254) and from Experimental Animal Committee of the University of Jyväskylä (permission numbers 19/5.6.2001 and 44/8.9.2003).

## Birds

Great tits were used as predators, since they are visual hunters and common in Central Finland in the wintertime. They are also bold in character and learn difficult tasks such as handling artificial prey items in captivity. The birds were caught from feeding sites with traps and ringed for identification. Altogether, 51 birds were captured and used in the study. After the experiments, the birds were released back into the wild at the location of their capture. They were housed individually in illuminated plywood cages, sized 64 cm×46 cm×77 cm. The daily light period was 11 h. The birds were offered sunflower seeds, peanuts, tallow and fresh water *ad libitum* and occasionally mealworms (larvae of *Tenebrio molitor*). Prior to the experiments, the birds were food deprived (ca. 2 h) to ensure motivation to search for the artificial prey.

## Artificial prey

The prey items were small pieces (approximately 0.1 g in weight) of almond glued between two 8 mm×8 mm pieces of paper. In making the prey items we used non-toxic glue (UHU Stic). One black-and-white signal was printed on both sides of the paper shell of the prey items. The palatable cryptic prey items comprised a cross symbol (X) that was also printed on the background on aviary floors (see “Aviaries”). The two different signals that were used as models and mimics were a square (■) and an asymmetrical star (★).

The prey items that acted as models were made highly unpalatable by soaking the almond as slices for an hour in a solution of 30 ml of water and 2 g of chloroquine phosphate (malaria drug Heliopar, henceforth ‘chloroquine’). The almond in the mildly unpalatable mimics was prepared the same way in a solution containing only 0.25 g of chloroquine. After soaking, the almond slices were dried in room temperature overnight before being cut up into pieces. This difference in chloroquine concentration translates to a difference in unpalatability to great tits (Lindström et al. 2006; Ihalainen et al. 2007). The cryptic palatable prey items contained untreated almond.

## Aviaries

The experiments were carried out in three aviaries. The large aviary was 3.5 m high with a floor area of 57 m<sup>2</sup>. The floor was covered with white A3 size paper sheets, which

were glued together and covered with adhesive plastic. There were 71 crosses in random position printed on each sheet and ten fake cryptic prey items, which were printed crosses, cut out of white cardboard and glued randomly on the sheet. The purpose of the fake prey was to make the background three-dimensional, so that the cryptic prey would not be too discernible from the background. The paper sheets formed a grid with 15 rows and 22 columns on the floor. There were wooden dividers (ca. 6 cm wide boards) placed between each row, designed to ease prey handling and movement of the birds. In the large aviary, there were eight perches at the height of 0.5 m for prey handling. The two small aviaries were both 2.4 m high with floor area of 13.5 m<sup>2</sup>. The floors of these two aviaries were covered similarly to the large aviary, but there were eight rows of ten paper sheets. These aviaries had two perches.

In experimental setups only one prey item was placed per sheet, so as to be able to identify the attacked items by their coordinates. During the experiments the birds were observed through a one-way window. Fresh water was always available in the aviaries.

## 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 piece of paper, 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 bird had to eat all items before the training progressed to the next phase.

Following the initial training period, birds were also trained to forage from the cross symbol background, and familiarised with the aviaries to ensure that they could utilise the perches, and would readily search for the artificial prey in the experiment. Foraging from the cross symbol background was trained in the small aviaries. We put three cryptic prey items and twelve completely white prey items on the floor so that they were in the groups of three (one cryptic group and four white groups). One prey item on the group was always on the divider, so that it was clearly visible. The second item was close to the board and the third was in the middle of the sheet. The bird had to find and eat all prey items. The three cryptic prey items were used since it is realistic to assume that the predators would have some experience of the cryptic prey before they first encounter aposematic prey (e.g. feeding in the nest). To familiarise the birds with the large aviary, several birds at a time were allowed to feed and remain overnight inside. The cross symbol background was replaced by transparent plastic during the familiarisation period, with peanuts, mealworms and sunflower seeds available on the floor.

The birds were always trained to handle the prey items prior to the background training, but for practical reasons they were familiarised with the large aviary in varied stages of their training.

### Testing the signals

Results about the mortalities of the co-mimics could be confounded if the birds preferred one of the warning signals to the other. Also, a difference in their visibility and therefore their risk of detection in the first place, could affect the results. Therefore, we first ran preference and visibility tests on the signals. Eight trained birds were used for the preference test and subsequently the same birds also performed the visibility test.

### Preference test

Prior to the test, the birds were familiarised with an experimental cage for at least an hour and during that time five white prey items were offered on a petri dish with brown paper on the bottom. The experimental cages were made of plywood and sized 50 cm×50 cm×70 cm. There was one perch and a water bowl in the cage. The floor was covered with brown paper. The birds were observed through a small net covered window on the cage.

Untreated almond in the preference test prey was weighed (0.085–0.100 g), so that the bird could not choose between prey based on the amount of food. The prey items were offered in pairs through a hatch, so that the star and the square signals were in turn on the left and on the right side of the petri dish. Three pairs were offered to the bird. The bird was allowed to eat both the prey items of the first pair, but from the following two pairs we allowed it to eat only its first choice before the other item was removed. Therefore, the test was conservative i.e., because the birds were not gaining positive experience on both signals, possible preferences were not lost as the test proceeded. The signal chosen by the bird was recorded.

With this test, no preference for one of the signals was found. The first choices of the birds were equal for the signals (four first choices for both the star and the square) and there was also no difference when the three choices of the birds were analysed (Wilcoxon signed-rank test  $n=8$ ,  $Z=-0.587$ ,  $p=0.557$ ). In the actual experiment, stars and squares were equally often the first conspicuous signal attacked ( $\chi^2_1 = 0.727$ ,  $p=0.394$ ) in the treatments with equal numbers of the signals presented (see below).

### Visibility test

In order to encourage the birds to eat prey showing any of the signals, we offered them prey items of all types in their

home cages prior to the visibility test. As the cryptic signal was included in the visibility test, it was offered along with the star and the square.

The visibility test was carried out in the large aviary. We placed 65 items of each prey type (the cross, the star and the square) in a random order on the aviary background, thus occupying 195 of the 330 sheets. All the prey items contained untreated almond. The bird was allowed to find 30 prey items and the numbers of each signal found were recorded. All handled prey was considered as detected, since we were measuring detectability, not acceptability or killing rate of the signals.

There was no difference in visibility between the co-mimic signals (on average 13.4 stars and 12.6 squares killed; paired samples  $t$ -test:  $t_7=0.505$ ,  $p=0.629$ ) but the cryptic signal was clearly less visible (on average 4.0 cryptic items killed) than the other two (paired samples  $t$ -test:  $t_7=6.818$ ,  $p<0.001$  for cryptic vs. star and  $t_7=9.529$ ,  $p<0.001$  for cryptic vs. square).

### Learning trial

The learning trial was run in the large aviary (see “Aviaries”) and all birds were trained as described in “Training of the birds”. During the trials the birds had to learn to avoid aposematic prey that they had no experience of, and to forage on the cryptic prey.

To study whether imperfect moderately unpalatable mimics could degrade learning, we manipulated the frequencies of the mildly unpalatable mimic and the highly unpalatable model. There were four treatments where the frequencies of the conspicuous prey were either 95 models and five mimics or 50 models and 50 mimics. We set the mimic frequencies to ‘very rare’ and ‘equally common as the model’ to efficiently test the benefits (or costs) of changing mimic abundance. This frequency manipulation was done both with the star signal as the model (and the square as its mimic) and with the square signal as the model (star as its mimic). In every treatment, there was an additional 100 palatable cryptic prey items available. As such, 200 of the 330 sheets were occupied. We used ten birds in one treatment and 11 birds in three treatments; 43 birds altogether. A prey item was considered killed when the bird opened the paper shell and tasted or ate the almond inside (i.e. gained experience of the unpalatability of the prey). The total number of each prey type killed was recorded. Each bird had to kill 30 prey items before the trial was terminated.

### Memory test

The memory test was conducted in the small aviaries a week after the learning trial. We presented 30 cryptic prey items and 15 squares and 15 stars on the aviary floor in a

random configuration. Sixty of the 80 sheets were therefore occupied. For half of the birds the star had acted as a model and for the other half, the square. All the prey items presented were palatable, so that we could observe how the birds' responses depended on their previous experience, without them having any opportunity to learn or refresh their memory during this test. The memory test reflects the "level of learning" i.e. the strength of the signal-taste association by measuring the birds' willingness to sample and consume the signalling prey again. The birds were allowed to kill 15 prey items, and the total number of each prey type killed was recorded.

### Data analysis

In addition to absolute numbers of models and mimics killed in the learning and memory trials, we also calculated a relative predation risk for the models and mimics separately: we divided the number of killed models/mimics by their expected risk if predation was random (the random expectation included cryptic prey). This takes into account that the co-mimics were presented to the birds in different numbers and thus had different probabilities of encounter. For example, when 100 cryptic items, 50 models and 50 mimics were presented and the birds killed 30 prey items in total, the expected risk for both models and mimics was 7.5 (and 15 for cryptic prey). The random expectation does not take into account the visibility difference of the two co-mimics and cryptic prey (i.e. likelihood of detection) but gives a reference point for comparing the risks of the models and mimics. Since the total number of prey presented was constant, the relative risk gives the same results as mortality (proportion of a certain prey type killed) only on a different scale where 1 denotes random predation. To analyse the learning trend, we similarly calculated relative predation risk for the aposematic prey (models and mimics combined) in the beginning and in the end of the learning trial i.e. within the first or last ten prey items killed (including cryptic prey). In that case the expected number killed was calculated for each bird separately based what was left in the setup for the bird to kill.

The data were analysed with SPSS 11.5 for Windows statistical package. All tests are two-tailed and non-parametric tests are used where the data did not meet the requirements of parametric tests.

## Results

### Learning trial

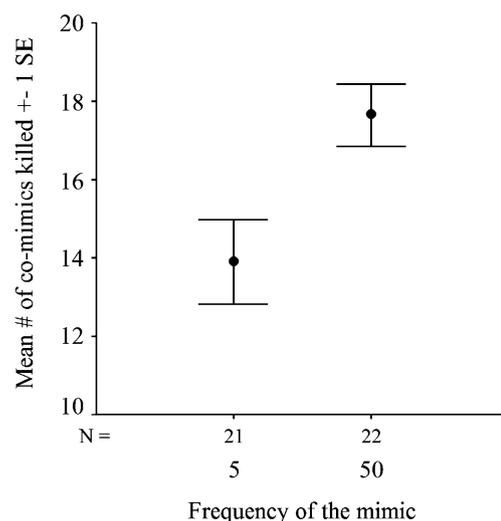
In absolute numbers, the birds killed more aposematic prey (i.e. models and mimics combined) when models and

mimics were equally common (ANOVA  $F_{1,39}=8.233$ ,  $p=0.007$ ; Fig. 1) compared to when mimics were rare. The signal treatment (star/square as a model) had no effect ( $F_{1,39}=1.666$ ,  $p=0.204$ ) and there was no signal  $\times$  frequency treatment interaction ( $F_{1,39}=0.772$ ,  $p=0.385$ ).

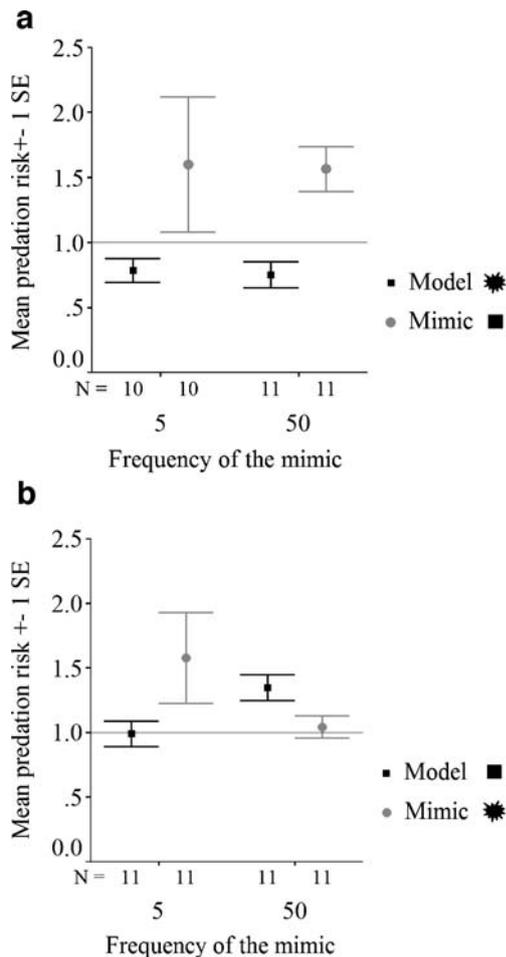
The increased predation on the two co-mimics could have indicated that the moderately defended imperfect mimics acted like Batesian mimics in the system or that increased variation in signal degraded learning. To test the effect of mimic frequency on mortalities of the co-mimics separately, we analysed the relative predation risks (see "Data analysis"). For the predation risk, there was an interaction between the signal and frequency treatments for the models (ANOVA  $F_{1,39}=4.077$ ,  $p=0.050$ ) showing that the effect of mimic frequency depended on the signals assigned to models and mimics. Therefore, signal treatments were further analysed separately.

Neither of the signal treatments showed a trend where both models and mimics would have had lowest mortality when the mimics are rare. When the star was the model signal, the frequency treatment did not affect the predation risk of the models (independent samples  $t$ -test  $t_{19}=0.225$ ,  $p=0.801$ ) or that of the mimics themselves (Mann–Whitney  $U$ -test  $n=21$ ,  $Z=-0.142$ ,  $p=0.918$ ) (Fig. 2a) but predation risk of mimics was constantly higher than of the models (paired samples test  $t_{20}=-3.027$ ,  $p=0.007$ ). In comparison, when the square was the model signal, risk of the models increased with increased mimic frequency (independent samples  $t$ -test  $t_{20}=-2.574$ ,  $p=0.018$ ) but the risk of the mimics decreased (Mann–Whitney  $U$ -test  $n=22$ ,  $Z=-2.171$ ,  $p=0.034$ ; Fig. 2b).

The importance of signal design for predation is evident in the 50/50 frequency treatments. The birds did not kill the



**Fig. 1** Mean number ( $\pm$ 1 SE) of co-mimics (models and mimics combined) killed during the learning trial in the two frequency treatments



**Fig. 2** Predation risk ( $\pm 1$ SE) of models and mimics in the two frequency treatments in the learning trial (a) when the star was the model signal and (b) when the square was the model signal. Black squares denote models and grey circles denote mimics. Reference lines show random predation

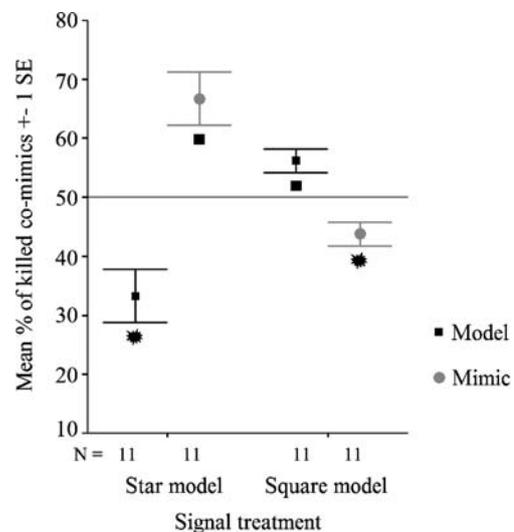
models and mimics in the same proportions as they were presented i.e., that within the total number of defended prey killed 50% would have been models and 50% mimics (Wilcoxon signed ranks test against test value 50; star models and square mimics both  $n=11$ ,  $Z=-2.402$ ,  $p=0.016$ , square models and star mimics both  $n=11$ ,  $Z=-2.380$ ,  $p=0.017$ ). They also did not attack the models and mimics according to their unpalatability level; the square was “overpredated” and the star “underpredated” irrespective of their relative unpalatability (i.e., whether they acted as models or mimics; Fig. 3). This indicates that the star was a more efficient warning signal than the square. This difference in signal design seems at least as important to the survival of the prey than the difference in unpalatability.

Learning took place in all treatments; at the end of the trial, the predation risk of defended prey (models and mimics combined) was lower than in the beginning (paired samples  $t$ -test all  $p<0.020$ ; i.e. within the last vs. first ten prey items killed, see “Data analysis”).

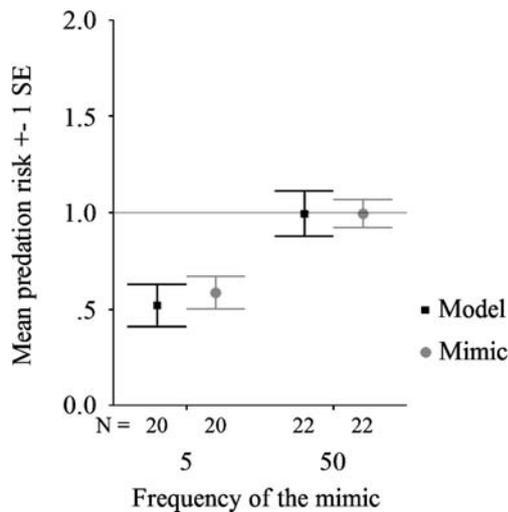
## Memory test

Predation risk in the memory test was calculated similarly to the learning trial above. Note that the signalling prey types are still called models and mimics according to the previous treatments, even though all prey was palatable in this test. Similarly, frequency treatment also refers to the frequencies of the co-mimics in the learning trial; here the models and mimics were presented in equal frequencies (15/15).

In the memory test, the birds from the 50/50 frequency treatment killed more models and mimics than birds from the 95/5 treatment (predation risk of models after In transformation ANOVA  $F_{1,38}=9.321$ ,  $p=0.004$  and of the mimics  $F_{1,38}=13.689$ ,  $p=0.001$ ; Fig. 4). In fact, predation of the co-mimics after the 50/50 frequency treatment was random (one-sample  $t$ -test  $t_{21}=-0.052$ ,  $p=0.959$  and  $t_{21}=-0.083$ ,  $p=0.934$  tested against random predation of 1.0 for the models and the mimics, respectively), whereas after the 95/5 frequency treatment, they were clearly avoided, i.e. killed less than randomly ( $t_{19}=-4.422$ ,  $p<0.001$  for the models and  $t_{19}=-4.826$ ,  $p<0.001$  for the mimics; Fig. 4). In this case, higher mimic frequency during learning translated to higher predation risk later on for both models and mimics. In contrast to the learning trial, the predation risk of the co-mimics did not show interaction between the frequency and signal treatments (for models after In transformation ANOVA  $F_{1,38}=0.654$ ,



**Fig. 3** Percentages ( $\pm 1$ SE) of killed models (black squares) and mimics (grey circles) of the total number of killed aposematic prey in the 50/50 frequency treatment in the learning trial. Percentages are shown for the two signal treatments separately: star and square symbols under the error bars indicate which signal was the highly unpalatable model and which one was the moderately unpalatable mimic in those treatments. Reference line shows equal predation on the models and the mimics



**Fig. 4** Predation risk ( $\pm 1$  SE) of models (black squares) and mimics (grey circles) in the memory test succeeding the learning trial. The two frequency treatments are shown separately but the signal treatments are combined. Reference line shows random predation

$p=0.424$  and for the mimics  $F_{1,38}=0.594$ ,  $p=0.446$ ) and there was also no main effect of the signal treatment ( $F_{1,38}=1.945$ ,  $p=0.171$  for models and  $F_{1,38}=2.728$ ,  $p=0.107$  for mimics) suggesting that signal design is more crucial during early learning or first encounters with predators. Since predation of models and mimics did not differ from each other within the frequency treatments (paired samples  $t$ -test  $t_{19}=-0.488$ ,  $p=0.631$  and  $t_{19}=0.000$ ,  $p=1.000$  for the 95/5 and 50/50 treatments, respectively) it seems that the birds now “pooled” the models and mimics despite visual imperfection and the previous inequality in unpalatability.

Birds from the 50/50 frequency treatment could have killed more co-mimics in the memory test simply because they received a lower dose of chloroquine during learning than the birds from the 95/5 frequency treatment where most of the aposematic prey were highly unpalatable models. As the dose cannot be accurately measured in this experiment, we made a rough estimate by assuming an eight-fold difference in the chloroquine concentration between the highly unpalatable and mildly unpalatable prey (there was an eight-fold difference in the solutions where the almond was soaked, see “Artificial prey”), and also assuming that the birds ate all of the almond inside the prey items. The dose (as a score, not a measure of concentration) for each bird was then calculated as  $8 \times$  number of killed models + number of killed mimics. The birds in the 50/50 frequency treatment received a smaller dose during learning (ANOVA  $F_{1,39}=11.879$ ,  $p=0.001$ ). However, the dose did not explain the behaviour of individual birds: a negative correlation between the dose estimate and the total number of co-mimics killed in the memory test would have shown that birds with presumably

more negative an experience with the defended prey (higher dose during learning) were less willing to attack them in the future. The correlation was negative (non-significant) only in one treatment ( $r=-0.511$ ,  $p=0.108$ ) and positive in the remaining three ( $0.273 \leq r \leq 0.783$ ,  $0.417 \geq p \geq 0.007$ ). Across all treatments, the estimate of received dose did not correlate with the number of signalling prey killed in the memory test ( $r=-0.024$ ,  $p=0.881$ ).

## Discussion

We did not find unambiguous evidence that a moderately unpalatable mimic could act as a Batesian-like mimic of a highly unpalatable model by degrading learning. During predator learning, the total number of killed co-mimics (models and mimics combined) did increase with increasing mimic frequency (Fig. 1) but the increase in predation did not affect the models and the mimics in the same way: there was a difference in signal efficacy, which had an effect on the model-mimic dynamics. The star appeared to be more effective a warning signal than the square (Fig. 3; see also Lindström et al. 2006; Ihalainen et al. 2008) even though the signals were novel, the birds did not show initial preference for either of them, and that they did not differ in visibility.

During avoidance learning, increasing the frequency of the mildly unpalatable imperfect mimic increased model mortality when the model had the weaker signal (square). Parallel to this the mortality of the mimic decreased (Fig. 2b). Although the effect of unpalatability cannot be excluded, this gives the impression that a uniform signal could be beneficial and a variable signal harmful (unlike in Rowe et al. 2004, Ihalainen et al. 2007) because both of the imperfect co-mimics were more likely to be taken when rare: the mimics had a higher risk at the frequency of 5 compared to 50, and the models had a higher risk at the frequency of 50 compared to 95 (see also Lindström et al. 2001). However, when the highly unpalatable model had the stronger signal (star), changing the model-mimic frequencies had no effect on the predation risk of either co-mimic (Fig. 2a). Instead, the highly unpalatable models had lower mortality than the mimics irrespective of frequency. The result is probably due to the model having “all the weapons”, both the stronger signal and stronger taste.

Neither of the signal treatments showed the trend that increasing the frequency of the mimic increases the mortality of both models and mimics themselves, which was demonstrated in an experiment where inexperienced great tits fed on mealworm larvae with artificial signals (Lindström et al. 1997) that represented aposematic models and their imperfect Batesian (fully edible) mimics. It should be noted, however, that in very low frequencies, small

differences in absolute numbers killed create large variation in relative measures of mortality. While such randomness can be an important factor in natural situations, it can also confound the comparisons of mortality in different frequencies. Nevertheless, frequencies and densities of unequally distasteful co-mimics have been manipulated in this system in other learning experiments (with equally efficient signals); Ihalainen et al. (2007) and Rowland et al. (2007) also did not find mortality costs due to increased number of moderately defended mimics (but see Speed et al. 2000; Skelhorn and Rowe 2006a).

As the trials progressed, birds switched from consuming conspicuous aposematic prey to cryptic prey in all treatments but this does not mean that learning would have been complete. The memory test showed differences in what the birds had learned and/or what they remembered from their experience with the co-mimics. Contrary to the learning trial the birds “pooled” the signals (showing that imperfect resemblance can be sufficient for effective mimicry) and thus the effect of the frequency treatment was more straightforward: higher mimic frequency during learning translated to increased predation of both models and mimics in the memory test (Fig. 4). This result could be seen as an indication that unequally defended co-mimics could have a Batesian-like relationship due to predator learning and memory (but see below). Note that even though the mortality of the signalling prey after the high mimic frequency treatment was on random level, the co-mimics still benefited compared to their high visibility.

The mechanism behind the result of the memory test could be that during learning the combined average unpalatability of the two co-mimics in the 50/50 frequency treatment was lower and/or there was more variation in the warning signal. In other studies with the same laboratory system, mere variation in signal or unpalatability had little effect on the mortality of aposematic prey (Ihalainen et al. 2007; see also Lindström et al. 2006). If we accept that “protection is a product of unpalatability and abundance” (Mallet 1999) the dose of chloroquine received by the birds during learning could have explained the observed result. On the treatment level, the crude estimate of dose was lower for the birds in the 50/50 treatment but it did not explain the behaviour of individual birds: there was no significant correlation to suggest that birds that received a lower dose during learning were more willing to sample the co-mimics in the memory test (but see Skelhorn and Rowe 2007). Furthermore, the estimate of dose may not correspond to the dose *ingested* by the birds because they typically only tasted the unpalatable prey items and sometimes seemed to taste-reject the models faster than the mimics (see also Skelhorn and Rowe 2006b). As an

alternative to the total dose of a received defence chemical it would be interesting to test whether the way it is received (a few prey items of high concentration vs. several items of low concentration) is important to learning and memory.

Our results suggest that the signal design of the prey might be at least as important during predators’ avoidance learning as relative unpalatability (Fig. 3). The birds did not use the co-mimic signals (which were reliable cues of the unpalatability level) in either trial (learning or memory) to simply attack the models and the mimics according to their taste. It has indeed been suggested (see Mallet 1999) that signal design could be one factor that makes a species “model” in a Müllerian system. However, the difference in mortality due to signal efficacy seemed to disappear with time as the birds gained experience about the prey; the signal treatments did not have an effect in the memory test where the birds also treated the models and the mimics in the same way (Fig. 4) unlike during learning when mimetic resemblance was partly uncertain. One could also speculate whether the details of signal design could affect the survival of prey when very rare (Fig. 2) or whether the mortality of rare mutations, although possibly high, is more random in that respect (Mallet 1990).

Overall, the present results show the dynamics of mimicry as a rather unpredictable interplay of signal design, taste and experience of the predators (see also Ihalainen et al. 2008). If a simple laboratory system can produce a complex co-mimic relationship, one could ask whether mimicry in the real world also shows “butterfly effects” where changing one factor can change the benefits of the mimetic relationship and the course of evolution considerably (see also Speed and Turner 1999; Kokko et al. 2003; Sherratt et al. 2004). Manipulating co-mimic frequencies whilst keeping their total density constant is an efficient and conservative way to study links between prey qualities and predator learning. How co-mimic species affect each other’s mortality in nature (at least at a given time) can also depend on the absolute densities of the species (see e.g. Owen and Owen 1984; Speed 1999; Sherratt et al. 2004; Rowland et al. 2007). The co-mimic relationship (parasitic/mutualistic) is a part of a broader question of “who mimics whom and why”. It is clearly recognised in the literature that the question may have a variety of answers (see Mallet 1999; Franks and Noble 2004; Darst and Cummings 2006). This is also suggested by the diversity found in nature such as sex-limited mimicry or polymorphism (e.g. Brown and Benson 1974; Nijhout 2003; see also Joron and Mallet 1998). From this perspective, our results about the sensitivity of the co-mimic relationship are not surprising but add to the view that there is more to mimicry than the simple principles of Bates and Müller.

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