[1] Co-mimics have a mutualistic relationship despite unequal defences

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In the first clear mathematical treatment of natural selection, Müller[1] proposed that a shared warning signal (mimicry) would benefit defended prey species by sharing out the per capita mortality incurred during predator education. Although mimicry is a mainstay of adaptationist thinking, there has been repeated debate on whether there is a mutualistic or a parasitic relationship between unequally defended co-mimic species[2–5]. Here we show that the relationship between unequally defended species is mutualistic. We examined this in a 'novel world'[6] of artificial prey with wild predators (great tit, *Parus major*). We kept the abundance of a highly defended prey ('model') constant and increased the density of a moderately defended prey ('defended mimic') of either perfect or imperfect mimetic resemblance to the model. Both model and defended mimic showed a net benefit from a density-dependent decrease in their per capita mortality. Even when the effect of dilution through density was controlled for, defended mimics did not induce additional attacks on the model, but we found selection for accurate signal mimicry. In comparison, the addition of fully edible (batesian) mimics did increase additional attacks on the model, but as a result of dilution this resulted in no overall increase in per capita mortality. By ignoring the effects of density, current theories may have overestimated the parasitic costs imposed by less defended mimics on highly defended models.

Since Müller's original statement of his mimicry theory in 1878 (ref. 1), müllerian mimicry has been explored comparatively[7], theoretically[8] and empirically[9–14]. Yet there remain ambiguities at the heart of the theory, leading to continued controversy and debate[5,8,15–17]. At its core, Müller's theory assumes that, to learn avoidance of prey with a particular visual signal, predators need to kill a fixed number of that prey. Hence, two distasteful species that have separate signals pay a higher mortality cost during predator learning than two species that, through mimicry, resemble each other. In contrast, Bates[18] proposed that perfectly palatable mimics could exist because they gained protection by exploiting predators' learned avoidance of defended model species. So although müllerian mimicry is considered to be beneficial to all mimetic prey, in batesian mimicry the model–mimic relationship is typically considered parasitic, because edible mimics degrade the predators' association of the shared signal with defence[19–21]. Despite the superficial simplicity of these mimicry theories, considerable confusion still exists in the literature, particularly surrounding the dynamics of an intermediate case between batesian and müllerian mimicry (in which both prey are defended but to unequal extents)[2,4,15,22,23]. Some authors argue that moderately defended species may act parasitically and dilute the protection of the better defended species[4], whereas others argue that the relationship is always mutualistic[5,8]. Furthermore, some theoretical models predict that changes in the total densities of unequally

defended mimetic prey can change their relationship from parasitism in low abundances into mutualism at higher abundances[3,17,24]

Despite the broad interest in these questions, there is no decisive experimental evidence that directly tests the opposing hypotheses. In this experiment we sought to resolve whether inequality in defence causes a mutualistic or a parasitic mimetic relationship when changes in prey densities are also incorporated. We tested the predation pressure imposed by predators that hunt visually (wild-caught great tits, *Parus major*) in a laboratory-based 'novel world'. In this environment the individual great tits were naive predators. We modified the densities of artificial mimetic prey that were unequally defended; a highly defended prey (here termed 'model') accompanied by a moderately defended prey (here termed 'defended mimic') or an edible mimic ('edible mimic'). In addition to this, we manipulated the degree of visual similarity of the mimic to the model (either 'perfect' or 'imperfect' mimicry, see Table 1). At the start of each trial, 60 edible cryptic prey were present as alternative prey (see Table 1). The highly defended model was either presented alone (120 prey at the start of each trial, model plus cryptic prey) or accompanied by 30 or 60 defended or edible mimics. Trials terminated when 50 prey had been 'killed' by a bird, simulating a predator with fixed-quota foraging. In addition, because mathematical simulations of mimicry often use time-based foraging[4], we analysed the data to simulate a time-limited forager (see Supplementary Information).

The birds responded differently to the highly and moderately defended prey items when these were presented alone, eating significantly more of the moderately defended mimic than the highly defended model (independent sample t-test, $t_{18} = -2.110$, $P = 0.049$). We found that increasing the number of defended mimics (treatments 1, 3, 4, 5 and 6) decreased the mortality of the models (Fig. 1a; general linear model (GLM), $F_{(2,44)} = 9.625$, $P < 0.001$), and this was true for both visually perfect and imperfect defended mimics (no effect of signal accuracy ($F_{(1,44)} = 0.283$, $P = 0.597$) or

Table 1 | Experimental setup

Perfect defended mimics					Imperfect defended mimics		Edible mimics			
Signal Type	Treatment				Signal Type	Treatment	Signal Type	Treatment		
	1	2	3	4		5	6		7	8	9
Cryptic	60	60	60	60	Cryptic	60	60	Cryptic	60	60	60
Model	60	–	60	60	Model	60	60	Model	–	60	60
Mimic	–	60	30	60	Mimic	30	60	Mimic	60	30	60

The types and numbers of prey presented in the experimental treatments are shown. An alternative edible prey was cryptic (a cross) as it matched the crosses on the aviary background where prey was presented (see Supplementary Fig. S1). Models always had a black square signal and were highly unpalatable. Mimics were either squares (perfect mimicry in treatments 3&4 and 8&9) or diamonds (imperfect mimicry in treatments 5&6), and were either moderately defended (treatments 2-6) or edible (treatments 8&9). Numbers in the columns correspond to the number of each prey type presented at the start of a trial.

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interaction between density and signal accuracy ($F_{(2,44)} = 0.026$, $P = 0.974$)). Hence the model benefited similarly from the presence of defended mimics regardless of whether the mimic's signal was perfect or imperfect (see also ref. 25).

The perfect defended mimics also had a decreased per capita mortality as their status as mimics changed from being non-mimetic at a density of 60 prey (presented without model; treatment 2) to being mimetic at a density of 30 or 60 mimics (with model treatments 3 and 4; Fig. 1b; $F_{(2,27)} = 11.432$, $P < 0.001$). Mimics did not show significantly lower mortality as they increased abundance from 30 to 60 prey (treatments 3–6; see Fig. 1b; $F_{(1,36)} = 3.509$, $P = 0.069$). Imperfect defended mimics showed higher mortality than the perfect mimics (Fig. 1b, blue bars; analysis of variance (ANOVA) $F_{(1,36)} = 5.843$, $P = 0.021$), indicating selection for perfect mimicry in the moderately defended mimics. There was no two-way interaction between mimic density and signal perfection ($F_{(1,36)} = 0.000$, $P = 1.000$; Fig. 1b).

Because the total density of prey increased when defended mimics were added to the setup, the probability of attack for a member of the population of models (the likelihood of being eaten by chance) decreased. To control for dilution we calculated the risk of attack for the models relative to that predicted assuming random predation (see Methods). This shows whether the models suffered increased attacks but to an extent that would have been masked in the measure of per capita mortality as a result of increased total density of prey. We found that the addition of defended mimics did not affect the mean risk of predation for models (no effect of mimic density ($F_{(2,44)} = 1.478$, $P = 0.239$), signal accuracy ($F_{(1,44)} = 0.256$, $P = 0.615$) or interaction between density and signal accuracy ($F_{(2,44)} = 0.022$, $P = 0.979$)). However, when we examined the learning process closely (see Supplementary Information) we found that the risk of predation for the model was lower at the end of the trial (Fig. 1c; last ten prey items killed) when paired with perfect compared with imperfect defended mimics ($F_{(1,36)} = 5.861$, $P = 0.021$; no effect of density ($F_{(1,36)} = 0.487$, $P = 0.490$) and no interaction between density and signal accuracy ($F_{(1,36)} = 0.325$, $P = 0.572$); see also ref. 13 and Supplementary Information for a detailed analysis of the learning process). This shows that, in addition to dilution, accuracy of mimicry is a source of benefit for the models. In terms of relative risk, the benefit of signal similarity was greater for the defended mimics (see Fig. 1b).

Because the effect of the difference in defence levels fits within the original müllerian framework (that is, the defended mimics benefit rather than harm the models), we tested whether our results match the quantitative predictions of Müller's original model (which assumes equality in defence between co-mimics). Müller assumed that if n individuals of mimetic species 1 and 2 are killed during predator learning, the number of each species taken is proportionate to their abundances (a_1 and a_2, respectively). We estimated two values of n: one was based on highly defended models alone (treatment 1) and the other on moderately defended mimics alone (treatment 2; see Supplementary Information). From these values of n the numbers of models and defended mimics killed (a_1 and a_2) could be predicted for the treatments in which both co-mimics were present. This allowed us to test whether the birds behaved as though all mimetic prey was of one defence level. We found that when mimics were added, the birds attacked more models than would be predicted if all prey in the model–mimic mixture were responded to as though they were highly defended (n estimated from models in Supplementary Table S1). The numbers killed were a better match to the prediction where n was based on the moderately defended mimics (see Supplementary Table S2). This can be explained by the mild taste of the mimics, which may have degraded learning (but see ref. 25), or by the tendency of predators to kill more aposematic prey (in absolute numbers) when prey are more numerous, even if all prey are highly defended[21]. The number of perfect mimics killed was intermediate, being not significantly different from either set of predictions (n based on models or defended mimics, Supplementary Tables S3 and S4); however, imperfect defended mimics (treatments 5 and 6 in Supplementary Tables S3 and S4) were taken at a higher rate than would be predicted if n were based on models. Despite some disproportionate costs incurred during learning, the result remains that mortalities decreased through dilution.

We next asked whether the strong dilution effect could extend to fully edible (batesian) mimetic prey. Abundant batesian mimics are generally considered to have a negative effect on their model's survival (see, for example, refs 18, 20, 21, 26, 27). However, it has been argued that edible mimics could prove beneficial to their models if they increase in abundance to the point of satiating the predator[15,17]. To test these opposing predictions, we increased the number of edible mimics in the setup while keeping the number of models constant (overall density increased with the addition of edible mimics).

We found that increasing the number of edible mimics had no effect on the mortality of models (Fig. 2a; GLM univariate ANOVA, $F_{(1,27)} = 0.015$, $P = 0.904$) but affected the mortality of edible mimics (Fig. 2b; GLM univariate ANOVA, $F_{(1,25)} = 44.619$, $P = 0.001$). The edible mimics had lower mortality when associated with models (Tukey post-hoc tests; 60 mimics alone versus 60 models plus 30
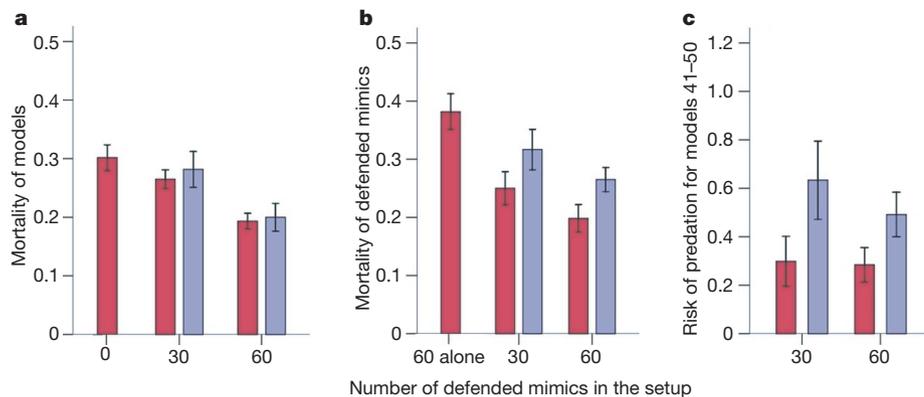

Figure 1 | Per capita mortality of models and defended mimics, and relative predation risk of models. a, Mortality of models when presented alone with 60 cryptic edible prey (mimics in setup 0) and when presented with 30 or 60 visually perfect (squares, see Table 1) defended mimics (red columns) or visually imperfect defended mimics (blue columns; diamonds, see Table 1). **b**, Mortality of visually perfect defended mimics (red columns) and visually imperfect defended mimics (blue columns) when presented alone with cryptic edible prey, or at a density of 30 or 60 with 60 models. **c**, The relative risk of predation for models in the last ten prey killed when paired with perfect (red columns) and imperfect (blue columns) defended mimics. All results are shown as means ± s.e.m.; $n = 10$ in all treatments.

mimics (treatments 7 and 8), $P < 0.001$, and 60 mimics alone versus 60 models plus 60 mimics (treatments 7 and 9), $P < 0.001$. However, the mortality of edible mimics was not affected by increases in their density from 30 to 60 ($P = 0.429$).

It does not necessarily follow that batesian mimics have no detrimental effects on their models. The absolute numbers of signalling prey killed (models and edible mimics together) increased with increasing numbers of edible mimics (Fig. 2c; GLM univariate ANOVA, $F_{(3,27)} = 5.67$, $P = 0.004$). To examine how relative predator choices changed with the addition of batesian mimics, we again calculated the mean relative risk of predation (see Methods). The relative risk for models increased with increasing number of edible mimics (Fig. 2d; GLM univariate ANOVA, $F_{(2,27)} = 3.57$, $P = 0.042$). This result also shows that the presence of edible mimics was detrimental to the models. Hence, in terms of relative risk of predation there is some evidence of a cost of batesian mimicry to the model, but the net effect of the edible mimics on per capita mortality is no change.

Thus, we found that the net effect of mimicry between unequally defended müllerian co-mimics was not parasitic. However, batesian mimics degraded the model's protection but this effect was more than compensated for through density-dependent dilution, which could be a real ecological benefit. As illustrated by the comparison of per capita mortality and relative predation risk, different measures can reveal different but equally relevant aspects of between-species dynamics (see also ref. 28). The comparison also raises the important question of predation pressure: if population sizes of mimetic prey change, how does the predator community react? Perhaps an increased density of defended prey does not attract additional predators as much as undefended prey (such as batesian mimics). The implication is that mimetic relationships between defended prey may be less sensitive than parasitic (batesian) relationships to changes at the community level (see also refs 29, 30). Our results clearly illustrate that the classic example of mimicry as an adaptation cannot be understood without explicitly considering the population and community context. Both the relative and absolute abundances of the species in the mimetic complex can have profound effects on mimetic dynamics in ways not clearly understood in the current mimicry literature.

METHODS SUMMARY

Ninety-seven great tits (*Parus major*) were pretrained to handle artificial prey items (pieces of almond in paper shells) and to forage in a 'novel world' aviary (see methods in ref. 25). There were nine treatments (Table 1); highly defended models were mimicked by, first, visually perfect, moderately defended mimics; second, visually imperfect, moderately defended mimics; or third, visually perfect, edible mimics (batesian mimics). Cryptic alternative prey was also presented (Table 1). High and moderate unpalatability of the prey items provided different levels of defence to the birds; they ate more of the moderately defended mimic than the highly defended model when these were presented alone with the cryptic prey (Table 1 and Fig. 1; see also refs 25, 28). The models and perfect mimics had a black square as a warning signal, whereas imperfect mimics had a diamond-shaped signal (Table 1). The numbers of models and cryptic prey were kept constant at 60. The models were presented either alone or accompanied by 30 or 60 mimics (moderately defended and edible; Table 1). Each bird was required to 'kill' (eat or taste) 50 prey items in the trial. We recorded the absolute numbers of all prey types killed and calculated the per capita mortality of each prey type by dividing the total number of each prey type killed during the trial by the number initially presented. We also calculated relative predation risk by dividing the number of each prey type taken by the predicted number that would have been killed assuming random predation (that is, in which prey types are eaten according to their frequencies). For example, because the predators were allowed to kill 50 prey items during the trial, the expected predation for models when presented with only cryptic prey was 25, whereas in treatment 3 the expected predation would be 20 models, 10 defended mimics and 20 edible crosses.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.
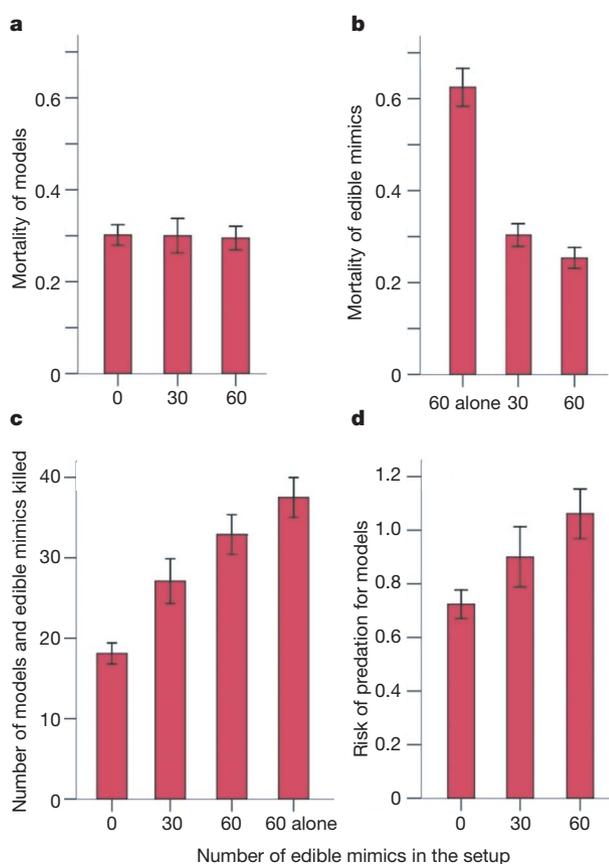
Figure 2 | Per capita mortality of models and edible mimics, total numbers of models and edible mimics killed, and relative predation risk for models. a, Mortality of models when presented alone with 60 cryptic edible prey (mimics in setup 0), and when presented with either 30 or 60 edible mimics. **b**, Mortality of edible mimics when presented alone with 60 cryptic edible prey (60 alone) or at a density of 30 or 60 with 60 models. **c**, The absolute number of models and edible mimics attacked when models were presented with 60 edible cryptic prey (mimics in setup 0), or when models were presented with either 30 or 60 edible mimics, and for comparison the treatment where edible mimics were presented alone with cryptic edible prey (60 alone, $n = 8$). **d**, The mean relative risk of predation for models when presented alone with 60 cryptic edible prey (mimics in setup 0) or with 30 or 60 edible mimics. All results are shown as means ± s.e.m.; $n = 10$ for all treatments, except where stated otherwise.

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METHODS

Ninety-seven wild great tits (*Parus major*) were caught at feeding stations around Konnevesi Research Station and were used as visually hunting predators in the experiment, which was conducted from January to March 2005 (permission was granted by the Central Finland Regional Environmental Center and by the Experimental Animal Committee of the University of Jyväskylä). Each bird was familiarized with a large experimental aviary (57.7 m^2 $\times$ 3.5 m height) and trained to open artificial prey items (see methods in ref. 25) and trained to forage from the aviary floor, which comprised a white background with black crosses printed onto it (see Supplementary Fig. S1). The artificial prey items used in the experiment (see Table 1) were made either highly or moderately unpalatable by soaking the almonds in a chloroquine phosphate solution for 1 h (2 g of chloroquine dissolved in 30 ml of water, or 0.25 g of chloroquine dissolved in 30 ml of water, respectively). These doses match those used in previous experiments[25,28]. The difference in concentration translated to a difference in palatability to great tits; the birds treated the highly and moderately defended prey items differently when they were presented alone, eating significantly more of the moderately defended mimic than the highly defended model (see the text and also refs 25, 28).

Learning experiment. We used three major conditions, split into nine experimental treatments which, comprised a highly defended model mimicked by, first, visually perfect, moderately defended mimics; second, visually imperfect, moderately defended mimics; or third, visually perfect, edible mimics (batesian mimics). The signal of the models and perfect mimics was a black square, whereas imperfect mimics had a diamond-shaped signal (Table 1). These signals do not differ in their visibility or efficacy as warning signals[25]. The number of models was kept constant at 60, and 60 edible cryptic prey items were also presented in all treatments as alternative prey. The highly defended model was either presented alone or accompanied by 30 or 60 mimics (see Table 1). Each bird was released into the experimental aviary, and the number and type of prey attacked were recorded. A prey item was noted as killed when the bird opened the paper shell and took a bite or ate the almond contained within the shell. Each bird was required to 'kill' 50 prey items before the trial was terminated.

Statistical analyses. In addition to the absolute numbers of prey killed, we calculated the per capita mortality of each prey type by dividing the total number of each type of prey killed during the trial by the number initially presented. We also calculated relative predation risk by dividing the number of each prey type taken by the predicted number that would be killed if predation were random, regardless of conspicuousness and defence levels. Predation is random when prey types are eaten according to their frequencies, taking into account the frequency of the cryptic prey. Because the predators were allowed to eat 50 prey items during each trial, the expected predation for models when presented with only cryptic prey was 25. In treatment 3, for example, the expected predation would be 20 models, 10 defended mimics and 20 cryptic edible crosses. Because the models and mimics were equally visible, and the number of less conspicuous cryptic prey was kept constant, the visibility difference between the cryptic and signalling prey does not affect the comparison of risks for co-mimics between the different densities. The data did not require transformation to satisfy requirements of parametric statistics. The data were analysed by GLM with Tukey post-hoc pairwise comparisons in SPSS v.13.0. Details of further analyses are given in the Supplementary Information.

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