

continental crust is thus sufficient to reproduce the extreme DUPAL compositions of RTJ MORB and 15% for the 39–41° E SWIR segment. Most of the MORB from the central, southeast and southwest Indian ridges has a less marked DUPAL signature that requires less than 4% of lower continental crust to be recycled into its source. The fact that recycled lower continental crust probably does not melt entirely decreases significantly the quantity of lower-continental-crust-derived melt required for a given contribution on the isotopic compositions. Indeed, incompatible elements such as Pb, Sr and Nd would be enriched in the melt derived from the lower continental crust and, because the melting degree remains high, mantle sulphides may be removed from the residue and platinum-group elements such as Os would also be enriched³³.

All of these features support the idea that delaminating of continental lithosphere, including the mafic lower continental crust, is responsible for the Indian upper-mantle isotopic anomaly. Some fragments may have sunk down to the OIB reservoir material and mixed with other recycled components, which would explain the particular compositions of Indian and South Atlantic OIB. Finally, recycling significant amounts of lower continental crust may help to explain the relatively andesitic composition of the continental crust²⁸. □

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The evolution of müllerian mimicry in multispecies communities

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Prey species that are unprofitable to attack often share conspicuous colours and patterns with other coexisting defended species^{1–6}. This phenomenon, termed müllerian mimicry^{2,3}, has long been explained as a consequence of selection on defended prey to adopt a common way of advertising their unprofitability^{7,8}. However, studies using two unpalatable prey types have not always supported this theory^{9–12}. Here we show, using a system of humans hunting for computer-generated prey, that predators do not always generate strong selection for mimicry when there are two unprofitable prey types. By contrast, we demonstrate that when predators are faced with a range of different prey species, selection on unprofitable prey to resemble one another can be intense. Here the primary selective force is not one in which predators evaluate the profitabilities of distinct prey types independently, but one in which predators learn better to avoid unprofitable phenotypes that share traits distinguishing them from profitable prey^{13,14}. This need to simplify decision making readily facilitates the spread of imperfect mimetic forms from rarity, and suggests that müllerian mimicry is more likely to arise in multispecies communities.

If a predator community needs to attack a fixed number of each distinct form of defended prey (such as those with stings or toxins) before it learns to avoid them, and if this pressure is significant, then there will be selection on unprofitable prey to resemble one another^{2,3,7}. Although field experiments have lent support to the idea that common forms of unpalatable prey are at a selective

advantage over rare forms of unpalatable prey^{15–17}, the precise mechanisms involved have seldom been investigated. Experiments with avian predators feeding on two distinct forms (occurring at frequencies of 1:9 and 9:1)¹⁰, and just one form (4%, 12% and 32% of total prey)¹¹ of artificial distasteful prey have found that unpalatable phenotypes have a greater per capita probability of attack when they are rare. However, similar experiments using garden birds⁹, domestic chicks⁹ and captive great tits¹² as predators found little or no evidence to indicate that the common form is a selective advantage over the rarer form. These findings contradict the traditional assumption that there should be selection towards uniformity because one colour pattern is easier to learn than two¹².

One reason for the above results may be that predators are not sufficiently confused to generate selection for mimicry when just two different forms are involved; they may learn rapidly to avoid both types of prey, regardless of their level of resemblance. It has recently been argued that there is likely to be far stronger selection on defended prey to adopt a common form of advertisement when there are multiple forms of prey available¹³. However, despite calls for experiments¹⁴, no study has investigated selection for mimicry when there are more than two unprofitable prey types. Natural communities often contain a wide variety of prey types that differ in their profitabilities and appearances; müllerian mimics themselves frequently participate in complex mimicry rings¹⁸. Here we used a system of humans foraging on computer-generated prey to examine the nature and intensity of selection for mimicry when there were

few forms of prey, and when there were many different types of prey available.

To evaluate the strength of selection for mimicry in simple systems containing relatively few prey phenotypes, we first conducted several related experiments (experiments 1 and 2a–c; see Methods). In experiment 1, human ‘predators’ were allowed to search a virtual environment in which they encountered individual prey items selected at random from populations of a profitable form of prey (mottled green, 40 items in total), and two unprofitable forms (mottled red and mottled blue, at nine combinations of frequencies that each totalled 40). As with previous studies^{10,11}, predators attacked more of the common form of the unprofitable prey available (Fig. 1a; number of blue attacked versus frequency of blue available $r_{38} = 0.42$, $P = 0.007$; number of red attacked versus frequency of red available $r_{38} = 0.47$, $P = 0.002$). This relationship has been explained as a consequence of predators not seeing enough of the rarest forms to complete their learning^{10,19}. However, in our study we observed how our predators behaved at each and every encounter, and noted that even rare unprofitable prey tended to be rejected by the end of the experiment (see Supplementary Table 1). This indicates that avoidance learning was largely complete, even when the unprofitable prey frequencies were relatively low. A more consistent explanation for the phenomenon may be that predators occasionally return to attacking the more common unprofitable type to assure themselves that all of that prey type were unprofitable, because there would be more to lose if some turned out to be profitable.

Despite the increase in number of unprofitable prey attacked with the frequency presented, the per capita ‘mortality’ of the unprofitable forms declined as their frequency increased (Fig. 1b). Overall, this observed predatory behaviour would be capable of generating selection for müllerian mimicry, with particularly rare forms of unprofitable prey selected to resemble common forms of unprofitable prey. Here we have elucidated selection at many more relative frequencies than have so far been examined in a single study, and can confirm, as Müller had anticipated^{2,3}, that the difference in survivorship between the rare and common forms was smaller when unprofitable prey types were similar in frequency. Unsurprisingly, significant selection for müllerian mimicry was not always evident at these intermediate frequency combinations (legend to Fig. 1b), and whether or not this was a consequence of low statistical power, it remains clear that selection under these intermediate conditions was at best weak.

In experiments 2a–c (see Fig. 2 for outline) we again investigated the strength of selection for müllerian mimicry in a simple prey community (one profitable and two unprofitable forms), this time evaluating the relative success of imperfect müllerian mimics. To do this we compared the survivorship of a distinctly coloured rare unprofitable prey (the ‘focal’ form) that was: plain, like the profitable form (experiment 2a); striped, like the more common unprofitable form (experiment 2b); or spotted so that it looked like neither the profitable nor the common unprofitable form (experiment 2c). Here, the mean per capita attack rates on each of these three forms in the three separate treatments did not differ significantly (Fig. 3, ANOVA $F_{2,27} = 2.966$, $P = 0.068$). Thus, even if selection occurred, it would not strongly favour the rare striped müllerian mimics in this simple community.

In our next experiments (experiments 3 and 4), we evaluated the strength of selection for müllerian mimicry in a more complex system with multiple forms of profitable and unprofitable prey present (Fig. 2). All of our computer-generated prey ‘species’ were distinct in appearance and were equally common (12 individuals of each). In experiments 3a–c, all of the non-focal unprofitable prey (six species) shared a common pattern element (a stripe) that was not exhibited by any of the profitable prey (six species). By contrast, in experiments 4a–c, a stripe was exhibited by three of the

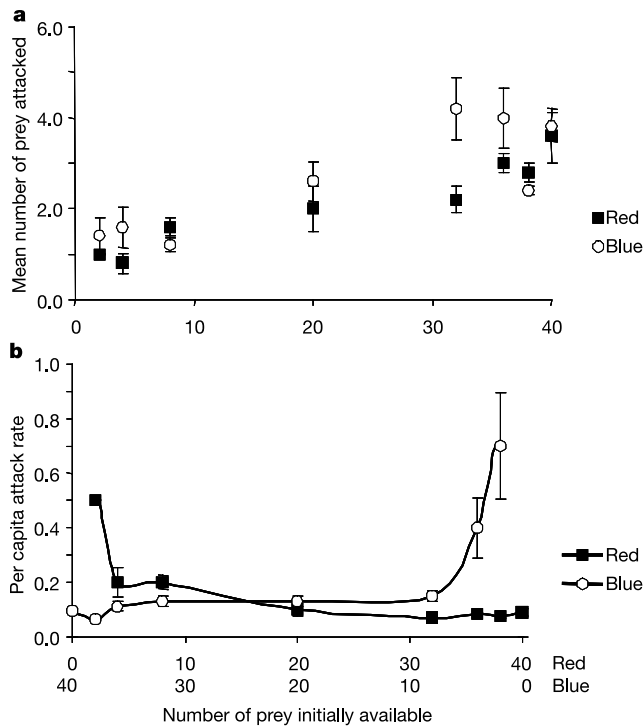


Figure 1 Results from experiment 1. **a**, The mean number of red and blue unprofitable prey attacked by predators (± 1 s.e.) in relation to the number of these prey items initially available; **b**, the implications of this behaviour for their mean per capita ‘mortality’ (± 1 s.e.). The proportion of red items attacked was significantly higher than that of blue items only when there were two red and 38 blue items (pairwise t -test on arcsin-transformed proportions $t_4 = 40.77$, $P < 0.001$). The proportion of blue items attacked was significantly higher than that of red when there were two, four or eight blue items ($t_4 = 4.58$, $P = 0.010$, $t_4 = 4.15$, $P = 0.014$, $t_4 = 7.915$, $P = 0.001$, respectively).






















Experiment	Profitable prey available	Non-focal unprofitable prey available	Focal unprofitable prey form(s) available
2	 72	 72	a  [0.15] 12
			b  [0.09] 12
			c  [0.14] 12
3	 12 12 12 12 12 12	 12 12 12 12 12 12	a  [0.25] 12
			b  [0.05] 12
			c  [0.13] 12
4	 12 12 12 12 12 12	 12 12 12 12 12 12	a  [0.28] 12
			b  [0.28] 12
			c  [0.18] 12
5	 12 12 12 12 12 12	 12 12 12 12 12 12	a  [0.54] 8
			b  [0.00] 4  [0.08] 8  [0.58] 4

Figure 2 A summary of experiments 2–5, in which human subjects foraged in a community of profitable and unprofitable prey. The prey forms had colours, with or without a single stripe or spot, as depicted. The numbers underneath each prey type refer to the number of that form available initially. Our analysis centred on the fate of a focal

unprofitable prey species that was magenta in colour and either plain, striped or spotted. In experiments 2–4, the focal unprofitable species was monomorphic; in experiment 5 it was dimorphic. The mean proportions attacked of each of the focal types are given in square brackets.

profitable species and three of the non-focal unprofitable species. When a stripe was reliably associated with unprofitability (experiments 3a–c), the focal prey species that carried the stripe had a far greater probability of surviving than the other forms of this species that did not (Fig. 3, ANOVA $F_{2,27} = 14.52$, $P < 0.001$; Tukey post hoc comparisons: striped versus plain, $P < 0.001$, striped versus spotted, $P = 0.018$). In these cases the focal striped prey were not only attacked far less frequently on first encounter, but they were also subsequently avoided more quickly than other forms (see Supplementary Fig. 2).

When no trait was reliably associated with unprofitability in these complex systems (experiments 4a–c), there was no significant difference in the proportions of these three different forms attacked in the separate experiments (Fig. 3; ANOVA $F_{2,27} = 1.84$, $P = 0.179$). In this case, predators continued to attack each form of the focal unprofitable prey for some time, generating low mean scores for these foragers (see Supplementary Fig. 3) and overall high prey mortality. The observation that striped prey had a greater survivorship in experiment 3b compared to 4b (ANOVA $F_{1,18} = 26.17$, $P < 0.001$) strongly suggests that predators were not simply learning to avoid all distinct unprofitable prey at equal rates. Instead, predators avoided attacking prey phenotypes at a rate related to the degree to which their traits could be categorized as unprofitable.

It has been argued that a predator's capacity to remember simultaneously the profitabilities of a wide range of prey types may be limited, and this may have an important influence on the nature of selection experienced by potential prey^{13,14}. To test this theory directly, we conducted a two-way ANOVA to compare the proportion of each focal prey type attacked in the simple (exper-

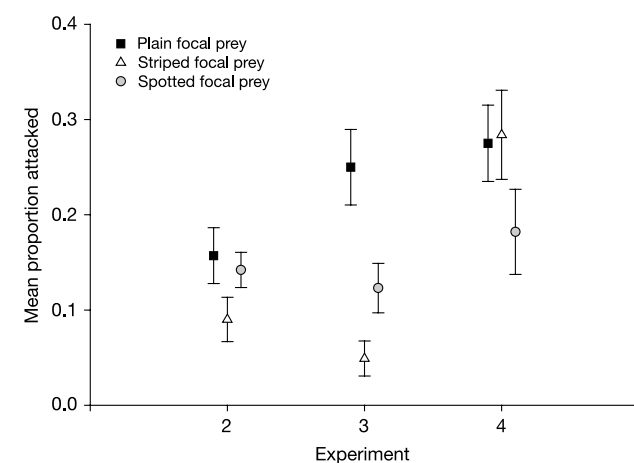


Figure 3 The mean proportions (± 1 s.e.) of each of the three forms of focal unprofitable prey attacked in experiments 2–4. An overall two-way analysis of variance on arcsin-transformed proportion of focal prey attacked revealed a highly significant interactive effect of experiment (2, 3 and 4) and focal form (plain, striped, spotted) ($F_{4,81} = 5.12$, $P = 0.001$) and highly significant main effects (experiment, $F_{2,81} = 11.91$, $P < 0.001$; focal form $F_{2,81} = 8.77$, $P < 0.001$).

iment 2) and the analogous complex (experiment 3) system. This analysis confirmed that system complexity had a significant influence on the proportion of each focal form attacked (interaction, $F_{2,54} = 3.48$, $P = 0.038$; experiment, $F_{1,54} = 0.061$, $P = 0.807$;

focal form, $F_{2,54} = 15.69$, $P < 0.001$), which seems to be driven by a combination of the increased vulnerability of the plain unprofitable form, and the decreased vulnerability of the striped (mimetic) form when the system was complex. Furthermore, given that the mimetic form was attacked at a significantly lower rate than both the plain and spotted forms when the system was complex but not when it was simple, we conclude that selection for mimicry can indeed be more intense when there are multiple species, at least under the specific conditions we have compared.

Taken together, our results suggest that there may be selection for unprofitable species in complex systems to maintain and enhance certain features (such as a colour or stripe in the right place) which happen to be shared by more unprofitable species than profitable species in a given area. To test whether an imperfect mimic could spread from rarity in a small population that contained a more common conspecific which lacked the mimetic trait, we conducted experiments 5a and 5b (Fig. 2). Despite its rarity, and the fact that it was seen at least twice by predators in all replicates, a rare form that shared a trait (stripe) with the other unprofitable species in the community had a far higher per capita survivorship than more common conspecifics that did not (experiment 5a, paired t -test on arcsin-transformed proportions $t_9 = 9.73$, $P < 0.001$). Similarly, when the striped focal form was more common than its plain conspecifics, its per capita survivorship was significantly higher (experiment 5b, $t_9 = 10.75$, $P < 0.001$). These results indicate the probable fate of intermediate phenotypes in the evolution of müllerian mimicry. If a distinctive mutant form of an unprofitable species arises, we might expect that it would suffer high mortality due to its unique appearance and extreme rarity. However, our results show that if the mutant shares simple signalling traits with more common unprofitable species, then it may well survive at higher rates than conspecifics lacking these traits.

The relative survivorship of each of our distinct focal forms differed according to the experimental context, which strongly suggests that foragers did not rely entirely on associative learning (as Müller had originally envisioned^{2,3}), but used simple rules to distinguish between profitable and unprofitable prey. Discriminative learning has been widely discussed in the psychological literature^{20–22}, but to our knowledge this is the first study to show that this type of behaviour can facilitate the spread of rare mimics with imperfect resemblance only, in a manner that is not apparent in simplified systems with relatively few prey types. As Fisher observed²³, “being recognized as unpalatable is equivalent to avoiding confusion with palatable prey”. Although Müller did not consider the appearance of profitable prey at all when making his arguments, such considerations may be essential to a full understanding of when and how müllerian mimicry evolves. □

Methods

All human ‘predators’ were visitors to the Page Break Coffee Bar situated within the MacOdrum Library at Carleton University. A total of 155 human predators (all volunteers) participated, of which 92% were non-biologists. Predators were not informed about the experimental aims and no subject was allowed to participate more than once.

The computer program was written in MS Visual Basic 6. The artificial foraging environment of each predator consisted of a grid of $n \times n$ cells. Prey (7×7 mm square and coloured/striped/spotted in a particular way according to type) were distributed within cells of this virtual grid, with no more than one prey item per cell. The predator saw only one randomly selected cell of the grid at a time (the position of the cell in the grid was not displayed) viewed in a square arena (148×148 mm) on the computer screen (see Supplementary Fig. 1). The background of the arena was comprised of a mosaic of 10% green and 90% white pixels. Predators changed cells in the search for prey by pressing a command button, causing a new cell to appear in the arena. When predators moved to a new cell that contained a prey item, they could either attack it (by clicking with the mouse cursor on it) or choose to move on without attacking it. On attacking a profitable prey item the predator gained a point and a high-pitched sound was made (which indicated profitability more effectively than using a score alone). On attacking an unprofitable prey item, the predator lost a point and a low-

pitched sound was made. Prey items that were attacked disappeared from the system. Predators were asked to forage for prey for five minutes in a way that would maximize their personal scores.

Experiment 1

Profitable prey (mottled green: 70% green, 30% white pixels, 40 items in total) and two forms of unprofitable prey (mottled red and mottled blue: 70% red and blue respectively, 30% white pixels, with a combined initial frequency of 40) were randomly distributed in a 10×10 grid. Overall, nine relative frequencies of red and blue unprofitable forms were presented (0/40, 2/38, 4/36, 8/32, 20/20, 32/8, 36/4, 38/2 and 40/0) and we allowed five different naive human predators to forage at each relative frequency (45 different human subjects in total).

Experiments 2–5

A total of 156 prey items were distributed in a 13×13 grid. Fifteen forms of prey were employed in total, consisting of blue, grey, green, red, yellow and cyan squares with and without a black stripe, and a magenta unprofitable prey type that was either plain, striped or spotted (see Fig. 2). The attack rate on the focal magenta unprofitable prey species was monitored in each of these experiments, which were all replicated ten times using different human predators (110 naive subjects in total).

Analysis

As our primary interest was in comparing the survivorship of focal prey under a given set of conditions, we used one-way analyses of variance (ANOVA) to test whether their per capita attack rates (arcsin transformed) differed according to their appearance (plain, striped, spotted). To compare the per capita attack rates (arcsin transformed) of the focal prey between experiments, we used a two-way ANOVA with experiment and focal form as fixed factors.

Using humans for foraging research

Müllerian mimicry is a taxonomically widespread phenomenon^{1–7}, so the foraging behaviours that generate it are likely to be exhibited by many different types of predator. Human subjects have long been used to test and refine ideas relating to predation^{24–26} and although humans represent visual foragers with a high capacity for learning and strategizing, they share with natural predators a finite capacity to process information^{21,27,28}. Indeed, recent work with human foragers has replicated the qualitative findings of earlier studies using great tits^{29,30}. The interpretation of our results does not depend on any behavioural traits that are unique to humans, and we hope these findings justify and inspire further work into müllerian mimicry evolution in complex communities using non-human predators.

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Hox cluster disintegration with persistent anteroposterior order of expression in *Oikopleura dioica*

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Tunicate embryos and larvae have small cell numbers and simple anatomical features in comparison with other chordates, including vertebrates. Although they branch near the base of chordate phylogenetic trees¹, their degree of divergence from the common chordate ancestor remains difficult to evaluate. Here we show that the tunicate *Oikopleura dioica* has a complement of nine *Hox* genes in which all central genes are lacking but a full vertebrate-like set of posterior genes is present. In contrast to all bilaterians studied so far, *Hox* genes are not clustered in the *Oikopleura* genome. Their expression occurs mostly in the tail, with some tissue preference, and a strong partition of expression domains in the nerve cord, in the notochord and in the muscle. In each tissue of the tail, the anteroposterior order of *Hox* gene expression evokes spatial collinearity, with several alterations. We propose a relationship between the *Hox* cluster breakdown, the separation of *Hox* expression domains, and a transition to a determinative mode of development.

Hox genes are involved in establishing morphological identities along the anteroposterior axis of bilaterians and cnidarians². Phylogenetic analysis suggests that the ancestor of all bilaterians had at least seven *Hox* genes—five anterior, one central and one posterior, according to the nomenclature of ref. 3—grouped in a genomic cluster where the gene order correlated with sequential expression along the anteroposterior axis. Both *Hox* gene sequences and the *Hox* cluster evolved in distinct animal lineages, with occasional cluster splits in invertebrate protostomes (*Drosophila*, *Caenorhabditis*), and gene losses^{4,5}. The significance of these discrete alterations in terms of developmental changes is a challenging enigma. Major gains of *Hox* genes coincided with the evolution of chordates, including the multiplication of entire clusters in vertebrates and an increment in the number of posterior paralogues up to six in vertebrates and cephalochordates^{6–8}. Recent sequencing in the tunicate ascidian *Ciona intestinalis*^{9,10} revealed only three posterior genes, which might equally represent a gain or a loss of genes. *C. intestinalis* also has only one central gene, probably reflecting a secondary loss of two genes, and its *Hox* cluster is split into five segments.

To gain further insight into the evolution of tunicate *Hox* complements, we identified the *Hox* genes of *O. dioica* and studied their expression and genomic organization. *O. dioica* belongs to the appendicularians, one of the three classes of tunicates. We recently showed that *O. dioica* has a very small (60–70 megabases), compact genome (one gene every 4 kilobases (kb))¹¹. Unlike ascidians, appendicularians conserve a chordate tail complex during the entire short life cycle (4 days at 20 °C in *O. dioica*). PCR cloning with degenerate primers, and whole-genome shotgun sequencing of both outbred and inbred populations (Supplementary Fig. S1), revealed nine candidate *Hox* genes. Full-length complementary DNA species were cloned for each of them, and phylogenetic analyses indicated that *Oikopleura* has three anterior *Hox* genes (*Hox1*, *Hox2* and *Hox4*) and six posterior *Hox* genes (*Hox9A*, *Hox9B*, *Hox10*, *Hox11*, *Hox12* and *Hox13*) (Fig. 1). Therefore, *O. dioica* and *C. intestinalis* share the same number of *Hox* genes but have markedly different *Hox* complements. *O. dioica* has lost the *Hox3* paralogue and all central genes, whereas *C. intestinalis* has probably lost some central genes. Either the posterior genes have been independently amplified in the *Oikopleura* lineage or a chordate ancestor already had a full complement of posterior *Hox* genes, which was subsequently reduced in the *Ciona* lineage.

We studied the expression pattern of each *O. dioica* *Hox* gene by *in situ* hybridization (see Methods) at 2.5 h after fertilization (tailbud stage), at 4 h (hatched tadpole) and at 6 h (mid-organogenesis). Here, we focus on the 4-h expression patterns (Fig. 2a), which were essentially identical to those seen at 2.5 h and were mostly concentrated in the tail. During late organogenesis, expression patterns evolve further and gradually include additional regions in the trunk/head. The tadpole tail consists of an epidermis, the 20 cells of a notochord, two rows of eight round muscle cells located dorsally and ventrally, and a nerve cord (about 60 neurons and support cells) placed on the left side of the notochord. Taken together, the expression patterns showed similarities to and important differences from those of other animals (Fig. 2b). Each tissue was the site of expression of only a subset of the nine *Hox* genes. Conversely, each *Hox* gene was expressed in only a subset of the four tail tissues, and in extreme cases in a single cell. Overall, the expression domains of distinct *Hox* genes overlapped only weakly, except in the epidermis, and most hybridizing cells expressed a single *Hox* gene. Within this partitioned expression, the sites of expression along the anteroposterior axis showed correlation with the order of the *Hox* paralogues. There were, however, several deviations from the expression collinearity, as defined by a perfect order of the anterior expression limits (with *Hox2* as the classical exception in vertebrates and in