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Ecological factors influencing the evolution of insects' chemical defenses

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How insect defense chemicals have evolved has remained relatively understudied, compared with the evolution of aposematic signals of such defenses. Because there is mounting evidence that chemical defenses can generally be expected to be costly, understanding the evolution of such defenses and their maintenance in the face of the potential for automimicry (signaling by individuals that do not invest in defense) is nontrivial. One potential explanation is that chemically defended insects suffer less from predation than those that do not invest in chemical defenses. Here, we use a series of models to explore aspects of the evolution of such costly chemical defenses. Our models predict that investment in costly defenses can occur across a wide range of predation intensities; however, if predation intensity is low, then the defense has to be very effective to be selected, unless the defense is very cheap. Furthermore, the evolution of antipredatory defenses will be relatively insensitive to the severity of any mechanism, whereby prey pay a cost every time they use their defense against an attacking predator even if they survive the attack, but sensitive to the form of the relationship between initial investment in constituting the defense and survival benefit. Once defense becomes common in the prey population, prey may get a frequency-dependent benefit if predators learn to avoid prey of this type after several attacks. Finally, we predict that increasing the rate of avoidance learning by predators encourages reduced investment in antipredatory defenses by prey. The potential for these predictions to be tested empirically is discussed. *Key words:* aposematism, predation, secondary defenses, survival, taste rejection, toxins. *[Behav Ecol]*

Many prey species possess chemicals that render them toxic and/or distasteful to their potential predators, and a multitude of different defensive compounds have been identified (e.g., Blum 1981; Eisner et al. 2005). The defense chemicals of plants and insects have been particularly well studied, and we are now beginning to understand the processes underlying the sequestration and/or synthesis of many defensive compounds (e.g., Blum 1987; Nishida 1994; Gilsan King and Meinwald 1996; Nishida 2002; Laurent et al. 2003). The question of how grazing by herbivores has influenced the evolution of plant toxins has also received considerable attention (e.g., Pasteels and Rowell-Rahier 1992; Burgess and Chapman 2005). However, in stark contrast, the question of how predation has influenced the evolution of insect defense chemicals has remained relatively understudied, perhaps because researchers have focused on understanding the signaling of defense to predators (aposematism) rather than the evolution of the defense chemicals themselves (e.g., Sillén-Tullberg 1985; Guilford 1986; Roper and Redston 1987).

The evolution of insects' chemical defenses may in some cases be relatively easy to understand because it seems that some defenses appear to involve the bearer in no measurable cost (Bowers 1988; Kearsley and Whitham 1992). However, in other cases, chemical defenses can be costly: these costs can be incurred by females investing time searching for suitable host plants on which to lay their eggs or by individuals growing more slowly because resources are required to metabolize, store, or synthesize toxins in a way that prevents autotoxicity (Zalucki et al. 2001). Experimental evidence suggests that synthesizing or secreting defense chemicals can both slow larval growth (Rowell-Rahier and Pasteels 1986; Dobler and Rowell-Rahier 1994; Zalucki et al. 2001) and lead to a reduction in the final size of adult insects (Cohen 1985), which in turn may reduce reproductive success.

The evolution of costly defenses can also be explained when the costs of defenses can be offset because the defensive agent has other functions. For example, a toxic chemical defense can also function as an energy store (Kearsley and Whitham 1992) or as an aid to the bearer's own ability to subdue prey (as commonly utilized by spiders, Escoubas 2006). However, in some species at least, costly chemical defenses appear to have no function other than as antipredator adaptations. When this is the case, it is difficult to understand the evolution of insect defenses unless there are individual benefits to investing in defense chemicals. This is because in the absence of individual survival benefits, individuals that do not invest in defenses (automimics) would have a selective advantage over those that do (automodels) because they do not pay the costs associated with chemical production (Guilford 1994). As a result, we would expect automimics to have higher reproductive success than automodels (at least initially, when they are uncommon in the population) and increase in frequency in the population. This makes it difficult to understand how costly chemical defenses have evolved and been maintained because investment in costly defenses does not appear to be an evolutionarily stable strategy.

One potential explanation is that chemically defended individuals suffer less from predation than those that do not invest in costly chemical defenses (Guilford 1994). However, chemical defense often cannot be detected prior to attack, meaning that in order for chemically defended individuals to suffer less from predation than visually similar undefended individuals, they must be more likely to survive predatory attacks. Although there is now some evidence that aposematic insects often survive predatory attacks relatively unharmed (Järvi et al. 1981; Wiklund and Järvi 1982; Sillén-Tullberg

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1985) and that predators selectively reject prey based on their chemical content (Gamberale-Stille and Guilford 2004; Skelhorn and Rowe 2006a, 2006d), it is currently unclear under what ecological circumstances such differences in survival would allow costly chemical defenses to evolve. Here, we use a series of models to explore aspects of the evolution of such costly chemical defenses.

There is growing body of evidence that avian predators can use taste to discriminate between prey items based on their level of chemical investment, selectively eating fewer defended individuals (Gamberale-Stille and Guilford 2004; Skelhorn and Rowe 2006a, 2006d). Furthermore, in some species, individuals' color signals accurately reflect their level of chemical defense (Bezzerides et al. 2007), meaning that birds may be able to use these signals to discriminate between automodels and automimics. This mechanism could provide selective pressure to adopt defense. However, there may be selective pressures acting in the opposite direction: the defense can be costly (Cohen 1985; Rowell-Rahier and Pasteels 1986; Dobler and Rowell-Rahier 1994; Zalucki et al. 2001), may not be 100% effective in allowing the prey to survive an attack (Järvi et al. 1981; Wiklund and Järvi 1982; Sillén-Tullberg 1985), and even if the prey survives its fitness may be reduced by injury or the energetic cost of escape. Below we construct a simple mathematical model to explore how these different selection pressures interact.

MODEL 1: INVESTMENT IN IMPERFECT DEFENSE

Model description

We assume that an undefended organism is killed if it is attacked by a predator. We assume that predators attack at rate A attacks per unit time. Implicit in this is the assumption that predator behavior is fixed and unchanging over a prey generation, and thus, predators do not (e.g.) learn to avoid the prey over the course of a prey generation. We further assume that a prey individual must survive for a time t before being able to reproduce. If it does survive for that time, then it has fecundity $F_{\max}$. We characterize the fitness of an undefended individual f_u as its probability of surviving long enough to reproduce multiplied by its fecundity.

Following McNamara and Houston (1992), this is simply given by

$$f_u = F_{\max}\exp(-At). \tag{1}$$

Let us now consider the fitness f_d of a defended individual. Let us assume that investment in defense costs a fraction c of fecundity and that such investment would lead to a probability of surviving an attack S. Then,

$$f_d = (1-c)F_{\max}\exp(-At(1-S)). \tag{2}$$

Notice that the cost of adopting a defense is experienced as a reduction in reproductive potential contingent on survival to the reproductive stage. This cost is fixed and is paid no matter how many attacks the individual experiences, even if that number is zero. The benefit of the defense is increased likelihood of surviving to reach the reproductive stage due to an increased chance of surviving any attacks that the individual experiences.

Model predictions and interpretation

We are interested in finding the value of S (S_c) for which $f_u = f_d$ because values of S above this critical value will select for evolution of the defense. From Equations 1 and 2, it is easy to show that

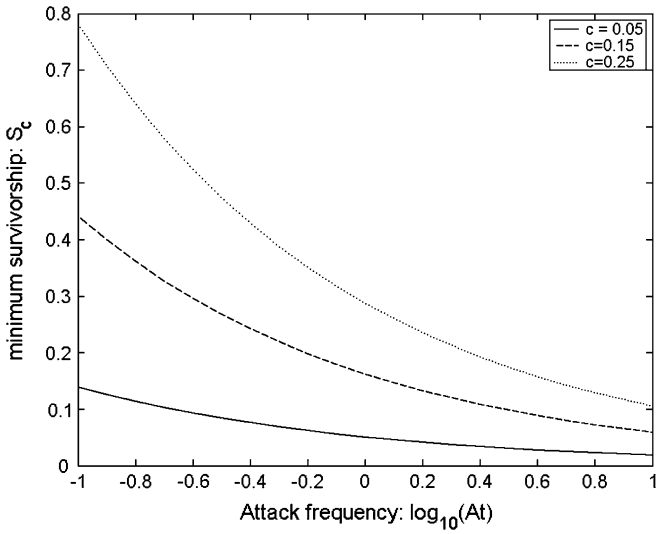

Figure 1
The predictions of model 1 found by solving Equation 3. The x axis describes increasing mean number of attacks over a lifetime, ranging from 0.1 on the extreme left up to 10 on the extreme right.

$$S_c = \left(\frac{1}{At}\right)\ln\left(\frac{1}{1-c}\right). \tag{3}$$

The behavior of S_c as a function of the parameter values is shown in Figure 1. We can see that if attack rate (A) is high and/or the cost of defense (c) is low, then defense need give very little advantage in probability of surviving an attack in order to be beneficial. For example, at the extreme right of the graph, a prey item is likely to get attacked 10 times on average over generation time t. The probability of escaping attack by chance is low, and so investment in defense is strongly selected for, even if this investment is so costly as to reduce fecundity by 25% and confers only say a 15% chance of surviving an attack. Conversely, at the extreme left-hand side, the chance of avoiding being attacked simply by chance is high, and so investment in defense is less favored. However, investment may still occur if the cost is relatively low. For example, we see that when there is on average only a 10% chance of an attack occurring (extreme left-hand edge of Figure 1) and defense only costs a 5% reduction in fecundity (solid line), then investment in defense will still be favored providing it gives at least a 14% chance of survival. However, if the cost creeps up to 15% (broken line), then the defense must be much more effective and confer at least a 43% chance of surviving an attack. Thus, this model predicts that investment in costly defenses can occur across a wide range of predation intensities; however, if predation intensity is low, then the defense has to be very effective at providing a substantial chance of surviving an attack to be selected, unless the defense is very cheap.

MODEL 2: COST TO SURVIVING AN ATTACK

Model description

It has generally been assumed in previous work (and model 1 of this paper) that the only cost to being attacked for a prey item is the risk of being killed in that attack. However, there are a number of reasons why being attacked might be costly, even if the prey individual survives. It may be that although it survives, the attack results in injury. For example, beak marks are often seen in the wings of aposematic butterflies that have

been sampled by avian predators (Smith 1979). These injuries could potentially alter fitness by influencing flight ability or attractiveness to potential mates, although this has not been investigated directly. Alternatively or additionally, surviving an attack may have an opportunity cost if time spent being attacked is time that could have been spent foraging. Further, avoiding death in an attack may cost energy or require expenditure of toxins that have then to be replaced at some expense. Hence, it seems reasonable to add some cost e to being attacked (even if the prey survives the attack). Notice that such costs are only incurred when an attack happens; if there are no attacks, then no such costs are paid. These "per-attack" costs are additional to the one-off costs (c) that were introduced in model 1. Thus, if the number of attacks faced is n, then if the defended individual survives to reproduce, we assume that fecundity is reduced to $(1 - c)F_{\max} - ne$. We assume that if this becomes negative, then the individual dies and has fitness zero. The constant c can be seen as the cost of constituting the defense and e as the (potentially recurring) cost that must be paid every time that defense is used against a predatory attack. If we assume that predation events are unpredictable and independent of each other, then it would be reasonable to model predation as a Poisson process with rate A, with the expected number of attacks during generation time t being At. The probability of a number of attacks n occurring is given by

$$P(n) = \frac{\exp(-At)(At)^n}{n!}. \quad (4)$$

The probability of undefended prey surviving to reproduce is simply the probability of no attacks occurring: $P(0) = \exp(-At)$, and thus, f_u is given by Equation 1 as before. We define n_c as the highest number of attacks that still allows a surviving individual to have a positive fecundity. This is given by the highest number such that

$$n_c e < (1 - c)F_{\max}. \quad (5)$$

The expected fecundity is calculated by multiplying the probability of a given number of attacks by the probability of surviving that number and the fecundity of a survivor and then summing these products over all numbers of attacks that allow positive fecundity. Thus,

$$f_d = \sum_{n=0}^{n_c} P(n)S^n((1 - c)F_{\max} - ne). \quad (6)$$

We are again interested in finding S_c such that $f_d = f_u$. S_c will be a function of At and c, as before, as well as $e/F_{\max}$. For notional convenience, we define

$$\varepsilon = \frac{e}{F_{\max}}. \quad (7)$$

Model predictions and interpretation

Model predictions for $c = 0.15$ can be seen in Figure 2; predictions for other c values are qualitatively similar. We can see that model behavior is relatively insensitive to the cost of surviving attack (ε), especially at high attack frequencies. An ε value of 0.21 reduces fecundity by 21% of that of an undefended individual for every attack survived and limits the number of attacks that can be survived to 4. This has almost no effect on the attractiveness of investment in defense when attack frequency is high. Under these conditions, the chance of avoiding any attacks by chance (when the expected number of attacks is 10 per prey individual) is so low that investment in defense is still worthwhile when using the defense that is costly because the odds of being attacked at least once but less than 4 times are much higher than the odds of avoiding ever being attacked. The effect of a cost of using the defense is more noticeable, if still only moderate, when the attack frequency is low. With low attack frequency, there is a substantial chance that an undefended prey will avoid any attacks, so investment in defense is only attractive if the costs of using the defense (ε) are low and survival benefits (S) are high. Increasing ε effectively increases the potential costs and so makes investment in defense less attractive, as can be seen in Figure 2. However, because this added cost is only paid if an attack occurs and attacks occur only infrequently, this cost is only paid in unusual circumstances. Thus, the effect of the value of ε is relatively undramatic even when at its strongest. Hence, the overall prediction from this model is that the evolution of antipredatory defenses will be relatively insensitive to the severity of any mechanism whereby prey pay a cost every time they are attacked even if they survive each attack.

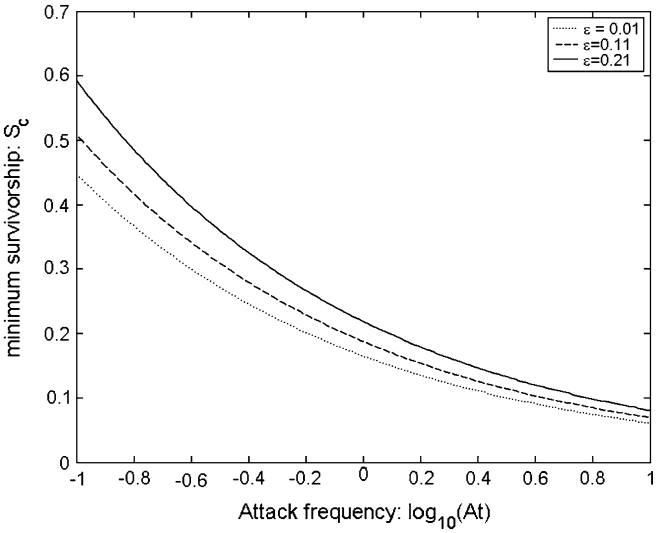

Figure 2
The predictions of model 2, assuming that surviving an attack still incurs a cost equivalent to 15% of maximum fecundity (i.e., $c = 0.15$).

MODEL 3: EVOLUTION OF STRENGTH OF DEFENSE

Model description

It is reasonable to assume that a greater level of defense not only requires a greater initial cost of constituting the defense (increasing c) but also leads to increased survival (increasing S) (Skelhorn and Rowe 2006a, 2006c). For simplicity, we will assume that the (per-attack) cost of using the defense (ε) is not dependent on the strength of investment, but S is given by

$$S = c^a S_{\max}, \quad (8)$$

where $S_{\max}$ is the probability of surviving attack when investment in constituting the defense is maximal (i.e., $c = 1$) and a describes how probability of survival increases with increasing investment. $a = 1$ suggests a simple linear relationship; $a < 1$ suggests a convex decelerating relationship of diminishing returns on investment, whereas $a > 1$ suggests a concave accelerating relationship. We are interested in finding C, the value of c that maximizes fitness $f(c)$. Fitness is evaluated using

an analogous formulation to that used in Equation 6 for model 2:

$$f(c) = \sum_{n=1}^{n_c}\left(\frac{\exp(-At)(At)^n}{n!}\right)(c^a S_{\max})^n F_{\max}(1 - c - n\varepsilon) + F_{\max}(1 - c)\exp(-At), \quad (9)$$

where n_c is the largest value such that

$$n_c\varepsilon < 1 - c. \quad (10)$$

We can simplify Equation 9 by noting that the factor $F_{\max}\exp(-At)$ is independent of c, so we need to find the value of c that maximizes

$$f(c) = \sum_{n=1}^{n_c}\left(\frac{(At)^n}{n!}\right)(c^a S_{\max})^n(1 - c - n\varepsilon) + (1 - c). \quad (11)$$

Model predictions and interpretation

The model predictions for the optimal investment in constituting the defense (optimal value of c) are shown in Figure 3 for $S_{\max} = 1$ and $\varepsilon = 0.05$; predictions for other parameter values are qualitatively similar. It is no surprise that the optimal investment in defense increases with increasing frequency of attack. What is more unexpected but very clear from Figure 3 is that the optimal investment can be very substantial, with individuals being prepared to invest so much in defense that their fecundity reduces by 50% in the case (extreme right of the figure) where the average number of attacks that an individual must survive in order to reproduce is 10.

Another clear prediction from this figure is that optimal investment level is sensitive to the form of the relationship between investment and probability of surviving an attack. In the case where this relationship is accelerating (e.g., $a = 1.5$), then small amounts of investment yield very little improvement in survival, and so we see (dotted line) that over a large range of attack frequencies, the best strategy is to make no investment at all in defense. When there is no initial investment in defense (i.e., $c = 0$), then a nonzero value of ε is still biologically justifiable. In this case, ε should be interpreted as the per-attack reproductive cost of surviving an attack. This could occur if flight from a predator uses energy stores that would otherwise be used in reproduction. Only after attack frequency exceeds a critical value is it worth investing very heavily in defense. Because of the accelerating nature of the relationship between investment and benefit, it is never optimal to invest small amounts in defense, and when investment is nonzero it is greater than for the other 2 relationships shown in this graph.

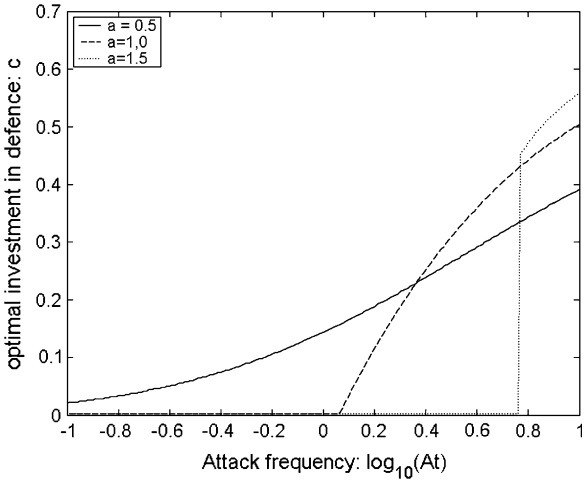

Figure 3
The predictions of model 3, when $S_{\max} = 1$ and $\varepsilon = 0.05$ for 3 values of the parameter (a) that controls the relationship between investment in defense and likelihood of surviving an attack.

Hence, if the relationship between investment and benefits is an accelerating shape, then we should expect to find that different species or populations follow one of the 2 strategies with individuals in a population either making very little investment in defense or making a large investment in defense. Thus, with this accelerating functional relationship between investment and probability of surviving, we would expect the natural world to be populated by some species that are very palatable and some species that are very toxic, with no intermediately toxic species.

The situation is unlike this if investment in defense yields a linear (dashed line) or decelerating relation (solid line) with probability of survival. In these cases, we see that a great variety of different levels of defense can be optimal, depending on the intensity of predation. So, if the relationship between investment in constituting the defense and probability of surviving an attack is nonaccelerating, then we would expect that the natural world would contain species with a great variety of different levels of toxicity, including palatable, mildly toxic, and highly toxic cases. It is no surprise that even at low levels of predation risk, the decelerating curve favors some investment in defense because even small levels of investment in defense can lead to a significant increase in likelihood of surviving an attack. Conversely, when predation pressure is high, then this decelerating relationship predicts the lowest investment in defense (compared with the other 2 relationships shown). This occurs because at high levels of investment, yet further investment leads to very little benefit in terms of increased survival. Hence, if the relationship between investment in defense and probability of surviving an attack is decelerating, then we would expect investment in defense to always be moderate and generally to be insensitive to predator intensity so that species would be generally similar in their level of defensive investment.

MODEL 4: FREQUENCY-DEPENDENT EFFECTS—AVOIDANCE LEARNING

Model description

Once defense becomes common in the prey population, prey may get a frequency-dependent benefit if predators learn to avoid prey of this type after several attacks. That is, an individual's probability of attack is now influenced by the distribution of individual defenses in the population that it is in. As the frequency of highly defended individuals in the population increases, average number of attacks on an individual over its lifetime declines. We study this using an individual-based simulation model.

We assume, as before, that prey must survive for a time t before reproducing. We assume that at the start of the generation, there are N prey and P predators. Each predator attacks at an initial rate α such that the initial expected per capita rate of predation (denoted A previously) is aP/N. The target of each attack is selected at random from the prey alive at that time. This randomness means that different replicate evaluations of the model will lead to a different order of victims of attack and so a different set of survivors at the end of a generation. Whether an individual survives or not is dependent on its own level of investment in defense, the levels of investment adopted by others, and luck (in the form of when it is

randomly selected for attack). However, although this luck will add noise to the selection process, the luck will even out over time, and so the long-term behavior of the model will be consistent between replicates (providing the population size is large enough).

Each individual is characterized by its level of investment in defense. For individual i, this level is denoted by c_i and is fixed for the duration of that individual's lifetime, implicitly it is genetically encoded. Investment in defense will influence an individual's survival, so we are interested in describing how the distribution of c values across the population changes over time (through luck, selection, and mutation during reproduction). For the results shown here, we assume that all individuals start off with no investment in defense (all c values are zero), although further simulations confirm that the final endpoint of evolution of our model is independent of the starting point.

The probability of the prey surviving a single attack is given by Equation 8 and is thus dependent on the investment in defense of that individual (c_i). Regardless of the success of that attack, the predator gains an amount of experience equivalent to c_i. A predator's experience is zero at the start of the generation, but when it reaches a value $c_{\max}$, then that predator desists from further attacks of that generation. Biologically, this encapsulates the idea that predators can only learn to avoid prey of this type by having aversive experiences after attacking some of them. The greater an attacked individual's investment in defense, the more aversive the experience, with the predator learning quicker from more aversive experiences (Skelhorn and Rowe 2006c).

The genotype of each prey encodes its personal value of investment in defense. If the prey survives until time t, then its fitness is calculated as $F_i = F_{\max}(1 - c_i - n_i\varepsilon)$. The next generation starts off with P-naive predators (i.e., with experience equal to zero) and a population of N offspring of the survivors of the previous generation. Each survivor's probability of being selected as the parent of a given member of the next generation being weighted by their fitness, then selected randomly according to these weights. Implicitly, we assume haploid genetics, so offspring inherit their parent's c value—subject to a small mutation drawn from a normal distribution with mean zero and standard deviation μ and resampling to ensure that selected values lie within the allowable range $(0, 1)$.

The prey population is allowed to evolve under this predation pressure, allowing us to observe the effect of variation in this pressure on the evolutionarily stable level of investment in defense. Figure 4 shows the mean and standard deviation of the distribution of c values across the population at the end of the final (1000th) generation. There is some noise because of the stochastic effects discussed earlier; however, it can clearly be seen that as $c_{\max}$ increases (and so predator avoidance learning is slower), investment in defense increases. The results shown are for parameter values $N = 2000$, $P = 20$, $t = 100$, $S_{\max} = 1$, $a = 1$, $\alpha = 0.5$, $\varepsilon = 0.05$, and $\mu = 0.02$, but extensive numerical investigations suggest that this general pattern of decreasing investment in defense as predators learn more readily (as $c_{\max}$ declines) is conserved regardless of the exact parameter values chosen. Essentially, more rapid avoidance learning (smaller $c_{\max}$) by predators reduces the per capita rate of predation and so (as can be seen in Figures 1–3) leads to a reduction in investment in defense. One might speculate that predator learning might induce investment in defense to reduce the number of attacks that each predator requires to complete its learning. However, this is a group-selectionist argument, and in the absence of very strong kin selection effects, such a strategy would be undermined by individuals that gain from the enhanced investment of others without investing as heavily themselves (automimics). One strong advantage of the simulation approach to exploring frequency-dependent effects is that we can explore the distribution of investment across the prey population and in particular look for evidence of automimicry.

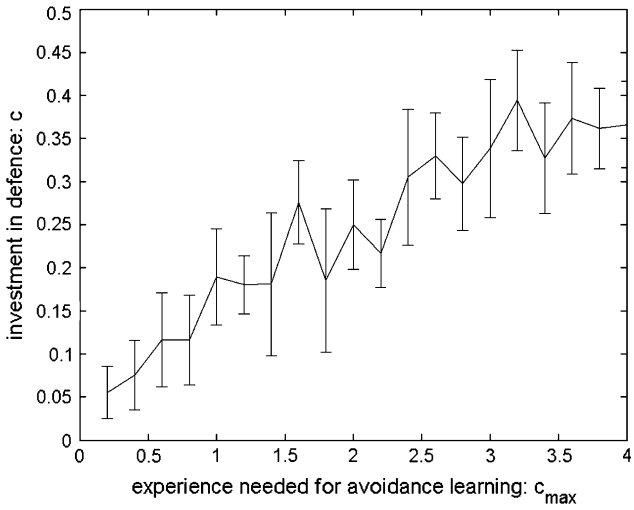

Figure 4
Predictions of the simulation model (model 4). Parameter values: $N = 2000$, $P = 20$, $t = 100$, $S_{\max} = 1$, $a = 1$, $\alpha = 0.5$, $\varepsilon = 0.05$, and $\mu = 0.02$.

Model predictions and interpretation

Figure 4 does show variation in investment across the population after a long period of simulated evolution. However, this variation should not be interpreted as evidence of automimicry. Automimicry would lead to within-population variation in level of defence driven by selection. However, the variation shown in our model predictions is driven by the inherent stochasticity in the system: inherent in the random selection of prey by predators, the probabilistic nature of surviving an attack, and the mutation involved in reproduction. We can see this stochasticity illustrated in the lack of pattern in the size of the standard deviations describing variability in investment across the population shown in Figure 4, whereas automimicry would lead to a pattern in this variation. In further simulations (not shown), we find that the average size of the standard deviation is affected by parameters that control mutation rate. Thus, variation across the population is induced by the effects of random chance, not by selection, and so there is no evidence of selection for automimicry. Again, extensive numerical investigations using different combinations of parameter values suggest that this effect is general for our model: increased rate of avoidance learning by predators encourages reduced investment in antipredatory defenses by prey, with this frequency-dependent learning failing to produce any evidence of automimicry in the prey population.

Another possible frequency-dependent effect of predator learning about defense is that predators that have previously encountered defended prey but have not learned to avoid them might still attack more circumspectly, increasing the chance of prey surviving an attack. Modification to the model (not shown) demonstrates that the effect of this mechanism is again to stimulate a reduction in investment in defense, but again extensive investigation of this model produced no evidence of automimicry.

GENERAL DISCUSSION

If an important mechanism allowing costly chemical defenses to evolve is predators selectively rejecting prey items based on

their level of chemical investment, then we can make certain predictions about the level of defense we would expect to evolve under different ecological circumstances. We found that selection can favor investment in costly chemical defenses over a wide range of predation intensities. As a result, we would expect to find defended insects in areas where predation intensity is low, as well as where it is high. However, when predation intensity in our simulations was low, the defense chemical had to be either very effective at enhancing survival or very cheap to produce in order to be favored by selection. We would therefore expect to see more interspecific variation in both the level of chemical defense and the cost of producing defense chemicals when predation pressure is high. This is because mildly defended insects, and costly defenses, are less likely to evolve when predation intensity is low. In addition, because a wider range of defenses can evolve when predation intensity is high, we may also expect a larger number of species to possess chemical defenses in areas where predation intensity is high.

Unfortunately, very little is known about how predation intensity correlates with the number of defended species or the type of defenses found in natural situations. Although it would be difficult to try to correlate the type of defenses found in areas with different predation intensities, it would be possible to look at the proportion of species that possessed defense chemicals. However, the predation intensity in a given area is unlikely to be stable over long periods of time, making it difficult to predict how the proportion of defended insects in the environment is likely to correlate with the current predation intensity.

Alternatively, experiments where artificial prey items are allowed to "evolve" under different levels of predation would enable us to test our predictions more directly. Such experiments have been performed in natural situations using pastry baits as prey: the baits are placed outside and garden birds are allowed to feed on them. After a period of time, the remaining baits are collected, and the number of each bait type placed out the following day is determined by the proportion of each type remaining. This process then continues until one bait type reaches fixation (Thomas et al. 2003, 2004). By manipulating the level of chemical defense of different bait types, we could look at whether predation intensity influences the likelihood of chemically defended prey reaching fixation. This could be achieved by choosing areas that naturally differ in their levels of predation or by manipulating predation intensities in a controlled manner in the laboratory.

Our models also predict that the evolution of chemical defenses will be relatively insensitive to the severity of any fitness costs associated with being attacked and released. We would therefore expect to find chemical defenses in prey with soft bodies (for which the fitness costs of being released after attack are likely to be high due to the ease with which tissues and vital organs can be damaged by contact with the predator), as well as prey with hard exoskeletons (for which these costs will generally be lower). In fact, this is exactly what we see in nature. Although many defended insects possess hard exoskeletons like that of millipedes and beetles, there are also some defended insects that have softer bodies such as caterpillars, butterflies, and many species of insect larvae (Eisner et al. 2005).

Interestingly, despite our predictions that the evolution of defenses is insensitive to the costs of being attacked and released, many chemically defended prey species appear to possess adaptations that increase the chance of surviving predatory attacks. For example, although sequestered chemicals may be stored systemically in body tissues (Brown and Francini 1990), many species store a large proportion of the chemicals in the integument and wings (Roeske et al. 1975; Brown 1984; Franzl et al. 1986; Nishida 1994). This may increase the speed with which predators perceive an individual to be defended and as a result reduce damage to the insect. Similarly, many insects have evolved complex methods to secrete defense chemicals onto their body surface (Eisner and Meinwald 1966), and some species, like the bombardier beetle, even spray potential predators with noxious chemicals before they are attacked (Eisner 1966). Based on our simulations, we would argue that these adaptations are not a prerequisite for the evolution of chemical defenses and are likely to have evolved either in conjunction with chemical defenses or after insects became chemically defended.

We found that the optimal level of investment in chemical defenses was sensitive to the form of the relationship between initial investment in constituting the defense and survival. If the relationship between investment and survival is accelerating, then we would expect to find a clear dichotomy in the level of defense among species, with some species investing little in defense and others investing substantially in defense. As a result, we would not expect to find species with intermediate levels of toxicity in the natural world. Conversely, when this relationship is either linear or decelerating, we would expect to see interspecific differences in the level of chemical defense. However, when the relationship between investment and survival is decelerating, although species will still vary in their level of investment in defense, we would predict that investment would generally be moderate. Thus, one important conclusion for our model is that in order to understand the evolution of defense, we must not only evaluate whether a given defense is costly but also identify how this cost is partitioned into one-off (constitutional) costs and recurrent costs paid as a result of each attack.

The situation in the natural world is likely to be far more complex than our models suggest. Although we know very little about the nature of the relationship between investment in defense and survival, the form taken by this relationship will probably be strongly chemical specific. This arises for a number of reasons, the speed with which predators perceive chemical defenses and the degree to which they can discriminate among different levels of a particular chemical are likely to influence the survival of defended insects (Skelhorn and Rowe 2006b). These factors are likely to be chemical specific, with the result that varying the concentrations of different chemicals may have very different effects on the survival of the insects that possess them. Similarly, the way that the concentration of a chemical influences its toxicity is also likely to be chemical specific because the range of concentrations over which potentially toxic compounds can be relatively innocuous varies among chemicals. The relative costs of increasing the concentrations of toxins could also vary among chemicals, depending on how the costs are paid. This situation is further complicated by the fact that many species appear to use complex mixtures of different chemical compounds in their defense (Pasteels et al. 1983). Taken together, this could result in very different relationships between investment in defense and survival across different chemicals.

This does not mean that it will be impossible to make predictions about the form taken by insect defenses in the natural world. On the contrary, it may be that our theory can provide several clear predictions. Now that it has been demonstrated several times that chemical defense can increase an individual's probability of surviving an attack, we very much need an empirically derived understanding of how variation in level of investment in constituting a defense (c in our model) affects probability of survival (S in our model). However, it seems likely that such empirical work will confirm that this relationship for a given species is of one of the 3 forms considered in our model (accelerating, decelerating, or linear). Once this

functional form has been characterized for a number of species (i.e., values of a in our model have been estimated for a number of species), then the following predictions derived from our models can be tested:

1. Insects that possess chemicals where the relationship between investment and survival is accelerating will be highly defended, and there will be little interspecific variation in the levels of these defenses.
2. Insects that possess chemicals where the relationship between investment and survival is linear could be either moderately or highly defended, and defenses could be found at different levels in different species.
3. Insects that possess chemicals where the relationship between investment and survival is decelerating will be moderately defended, and chemicals could be found in different levels in different species.

Variation in chemical content can be determined by measuring the amount of defense chemical that individuals contain/secrete and the concentrations of the chemicals present (e.g., Holloway et al. 1991). Measuring whether insects are moderately or highly defended is a little more difficult. However, it has recently been demonstrated that avian predators do not completely avoid toxic prey but continue to make strategic decisions to attack them at levels that allow them to maintain a "safe" toxin burden (Skelhorn and Rowe 2007). One potential method to determine levels of defense in insects is to determine how many prey individuals' predators are prepared to eat under tightly controlled laboratory conditions. Comparing this value across species will give some idea of relative toxicity and allow our predictions to be tested.

We also found that increasing rates of avoidance learning by predators encourages reduced investment in chemical defenses by prey. This is because more rapid avoidance learning reduces the likelihood of individuals being sampled, and as a result, individuals benefit less from investing in costly defenses. If increased avoidance learning does indeed encourage reduced investment in chemical defenses, as our models predict, then any factor that speeds avoidance learning may influence the degree to which insects invest in defense chemicals. Insects' visual signals are known to influence the speed of avoidance learning. For example, predators learn to avoid chemically defended prey more quickly when the prey are conspicuous than when they are cryptic (Roper and Redston 1987). As a result, the evolution of conspicuous warning coloration in chemically defended cryptic prey may result in reduced investment in chemical defense. Similarly, the presence of a bitter taste is known to speed the rate at which birds learn to associate a visual signal with the effects of a toxin (Franchina et al. 1997). We would therefore expect distasteful toxins to be found at lower levels than equally toxic, but palatable, toxins. Again, relative toxicity could be measured in the laboratory. When toxins are injected into mealworms, birds cannot taste them (Skelhorn and Rowe 2007). If insect toxins were injected into mealworms in this way, toxicity could be measured independently of distastefulness by measuring the number of worms birds were willing to eat under standardized laboratory conditions. In addition, if an already toxic insect species begins to produce an additional distasteful chemical, we would expect to see a reduction in the level of investment in the toxin.

Finally, we found that frequency-dependent learning by predators fails to produce evidence of automimicry. Recently, Ruxton and Speed (2006) demonstrated that automimicry was still logically possible if probability of surviving an attack increased with increasing investment in defense; however, this required one of a range of special circumstances. Specifically, these conditions were that the careful sampling of prey individuals necessary for enhanced survival of defended individuals was expensive (e.g., time consuming) for predators, predation pressure was sufficiently variable over time, predation pressure decreased as the average level of defense in the prey population increased, or there was a developmental or environmental constraint on the use of the chemical defense. We include none of these special circumstances in our model, and so our lack of observation of automimicry is exactly in accord with the previous theory of Ruxton and Speed (2006). Although logically plausible, the frequency of occurrence of such special circumstances in natural systems remains an open question.

It is important to remember that a full understanding of the evolution of chemical defense can only come about when integrating the issues discussed here with wider aspects of physiology, behavior, and life history. For example, there is an important theory demonstrating that the effectiveness of chemical defenses can interact with behavioral decisions involving aggregation (Sillén-Tullberg and Leimar 1988) or microhabitat selection (Speed and Ruxton 2005a). Further, although we assumed here that the costs of defense are experienced only at a terminal reproductive stage, it may be that the costs are felt more immediately (through, e.g., slowed growth), the consequences of this have been explored by Longson and Joss (2006). Models of the coevolution of defenses and aposematic signaling of such defenses have suggested that enhanced investment in signaling may be linked with reduced investment in the actual defense (Leimar et al. 1986; Speed and Ruxton 2005b); however, investment in defense need not necessarily lead to aposematic signaling (Broom et al. 2006; Puurtinen and Kaitala 2006).

In conclusion, if predators selectively rejecting prey based on their level of chemical investment have played an important role in allowing costly chemical defenses to evolve, we would expect to find defended prey in a wide variety of different ecological conditions. However, both the type of defense chemical and the level of investment in defenses are likely to be influenced by a number of factors: including predation intensity, the relationship between investment and survival, and the speed with which predators learn to avoid defended prey. We hope that the clear theoretical framework and explicit predictions presented here contribute to stimulating further empirical research to fully understand these issues.

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