BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2012), **87**, pp. 874–884.
doi: 10.1111/j.1469-185X.2012.00228.x

Why are defensive toxins so variable? An evolutionary perspective

Michael P. Speed[1,*], Graeme D. Ruxton[2], Johanna Mappes[3] and Thomas N. Sherratt[4]

[1] *Department of Evolution, Ecology and Behaviour, Institute of Integrative Biology, Faculty of Health & Life Sciences, University of Liverpool, Liverpool, L69 7ZB, UK*
[2] *School of Biology, University of St Andrews, Dyers Brae House, St Andrews, Fife, KY16 9TH, UK*
[3] *Centre of Excellence in Evolutionary Research, Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, 40014, Finland*
[4] *Department of Biology, Carleton University, Ottawa, K1S 5B6, Canada*

ABSTRACT

Defensive toxins are widely used by animals, plants and micro-organisms to deter natural enemies. An important characteristic of such defences is diversity both in the quantity of toxins and the profile of specific defensive chemicals present. Here we evaluate evolutionary and ecological explanations for the persistence of toxin diversity within prey populations, drawing together a range of explanations from the literature, and adding new hypotheses. We consider toxin diversity in three ways: (1) the absence of toxicity in a proportion of individuals in an otherwise toxic prey population (automimicry); (2) broad variation in quantities of toxin within individuals in the same population; (3) variation in the chemical constituents of chemical defence. For each of these phenomena we identify alternative evolutionary explanations for the persistence of variation. One important general explanation is diversifying (frequency- or density-dependent) selection in which either costs of toxicity increase or their benefits decrease with increases in the absolute or relative abundance of toxicity in a prey population. A second major class of explanation is that variation in toxicity profiles is itself nonadaptive. One application of this explanation requires that predator behaviour is not affected by variation in levels or profiles of chemical defence within a prey population, and that there are no cost differences between different quantities or forms of toxins found within a population. Finally, the ecology and life history of the animal may enable some general predictions about toxin variation. For example, in animals which only gain their toxins in their immature forms (e.g. caterpillars on host plants) we may expect a decline in toxicity during adult life (or at least no change). By contrast, when toxins are also acquired during the adult form, we may for example expect the converse, in which young adults have less time to acquire toxicity than older adults. One major conclusion that we draw is that there are good reasons to consider within-species variation in defensive toxins as more than mere ecological noise. Rather there are a number of compelling evolutionary hypotheses which can explain and predict variation in prey toxicity.

Key words: toxins and toxicity, predator, prey, biological diversity, ecology, evolution.

CONTENTS

* Address for correspondence (E-mail: speedm@liv.ac.uk).

I. INTRODUCTION

Of the many ways that organisms defend themselves against enemies, chemical defence is one of the most taxonomically and ecologically widespread (Eisner, Eisner & Siegler, 2005). Defensive toxins are used to prevent damage by enemies such as predators and parasites and are found in both simple and complex organisms including bacteria (Matz & Kjelleberg, 2005; Jousset *et al.*, 2009), fungi (Jousset *et al.*, 2009), animals (Blum, 1981) and plants (Zagrobelny, Bak & Moeller, 2008). The complex biochemistry and physiology required for the acquisition and maintenance of defensive toxins has been explored in some detail *via* the study of ecological chemistry (for example see reviews in Rothschild *et al.*, 1979; Blum, 1981; Brower, 1984; Bowers, 1990, 1992; Whitman, Blum & Alsop, 1990; Pawlik, 1993; Berenbaum, 1995; Hay, 1996; Trigo, 2000; Dobler, 2001; Nishida, 2002; Eisner *et al.*, 2005; Laurent, Braekman & Daloze, 2005; Zagrobelny *et al.*, 2008).

Amongst the many intriguing facts which emerge from decades of research in chemical ecology is the observation that defensive toxins are often variable within populations, both in terms of the total quantity of toxins and their chemical constituents (see for example Chapter 15 in Blum, 1981). Variability within and among prey populations in defensive toxins has been recognized repeatedly in the chemical ecology literature (e.g. Eisner, Alsop & Eisner, 1967; Pasteels, Gregoire & Rowell-Rahier, 1983; Holloway *et al.*, 1991; Alonso-Mejia & Brower, 1994; Vogler & Kelley, 1998; Goodger, Capon & Woodrow, 2002; Bezzerides *et al.*, 2007). The lubber grasshopper (*Romelia microptera*) presents an extreme example in which defensive secretions are so variable within populations that each individual animal studied has a different, idiosyncratic chemical profile (Blum, 1981; Jones *et al.*, 1986). In other species, toxin variation may be more simply characterised, for example the absence of toxicity within some individuals in an otherwise defended population (Brower, van Brower & Corvino, 1967; Jousset *et al.*, 2009). In a major review of insect chemical defences Bowers (1992, p. 219), for example, argued that in prey that sequester defensive toxins, a spectrum of palatability within prey populations is 'probably very common, if not ubiquitous within unpalatable insects' .

In evolutionary terms, phenotypic monomorphisms are relatively easy to explain as the outcome of directional selection. Explaining the persistence of phenotypic and/or genotypic variation within populations over long time scales is more challenging. Though the chemical ecology literature has made enormous progress in discovering the extent of variation in prey toxicity, there has in our view been less emphasis on providing and evaluating evolutionary explanations for this variation. Is it, for example, the case that inter-individual variability in toxins simply reflects the stochastic nature of the environments within which prey organisms exist and develop, such as the variable nature of plant secondary compounds available (Brower *et al.*, 1982; Malcolm & Brower, 1989; Bowers, 1992)? Or is the variability more systematic in its distribution, perhaps affected in predictable ways by frequency-dependent or disruptive natural selection?

Toxin variation among individuals can be quantified in a number of ways (Blum, 1981). For example the quantity or concentration of a single defensive chemical may vary among individuals. In addition, the nature of the defensive chemicals may vary, due to variation in their biochemical profiles. Our focus is to explore aspects of the evolutionary ecology of toxicity that can lead to persistent toxin variation. We deal with qualitative and quantitative variation separately building up from the simplest case, in which toxins are simply scored as present or absent, then looking at continuous variation in quantity of toxin, and finally looking at variation in the biochemical profiles of multi-compound chemical defences. Our starting points are the excellent earlier reviews on prey toxins and toxin variation (Brower *et al.*, 1968, 1982; Brower, 1984; Bowers, 1992; Pasteels *et al.*, 1995) which argue for an evolutionary perspective. Since these reviews were published there is greater theoretical and empirical understanding of the evolutionary processes that may determine the extent of toxin variation. Our purpose in this review is therefore to describe and evaluate alternative explanations for toxin diversity, whilst attempting to synthesise existing datasets with evolutionary concepts. In addition we propose some new ideas to explain the persistence of toxin diversity within populations.

To keep the scope of the review manageable we have deliberately focused on the explanations for variability within prey populations, placing less emphasis on the equally interesting question of among-population and among-species variations in toxicity. One important conclusion we reach is that toxin variation is not necessarily a reflection only of the environmental stochasticity which prey experience. Instead, natural selection may in different circumstances constrain or encourage toxin diversity.

II. VARIATION IN QUANTITIES OF TOXINS

We consider within-population variation in the quantity of toxins to come in two forms. The first, known as automimicry, is effectively Batesian mimicry within a single prey species, (in Batesian mimicry members of an edible mimic species copy the warning display of a distasteful model species). In automimicry a nontrivial proportion of an otherwise chemically defended population simply lacks defensive toxins, so the presence/absence of toxins in individuals can be effectively treated as a bimodal character (Brower *et al.*, 1967). In the second form, we consider the persistence of continuous between individual variation in the level of toxicity within a prey population.

Where toxicity is considered as a discrete trait, such as the presence/absence of chemical defence, then in essence the trait is polymorphic and we need to understand how the visible or invisible polymorphism is maintained. The general evolutionary explanations proposed to explain such co-existence are fundamentally no different from those invoked to explain polymorphisms in general and include frequency-dependent selection, heterogeneous selection and heterozygote advantage (Maynard Smith, 1998). When the variation is continuous, then it is in essence a 'massive polymorphism' (Cain & Sheppard, 1954; Moment, 1962) and an analogous set of explanations may apply.

(1) Automimicry in animals, plants and bacteria

One of the earliest studies of the ecological chemistry of plant-insect interactions (Brower *et al.*, 1967) revealed the phenomenon of automimicry. Brower *et al.* examined the relationship between toxic secondary metabolites present in milkweed plants, and those in the monarch butterfly (*Danaus plexippus*), which feed on milkweeds in its larval stage. Individual monarchs that fed as caterpillars on *Asclepias curassavica* became toxic and caused emesis in captive blue jays (*Cyanocitta cristata*). Individuals that fed on a different asclepiad plant (*Gonolobus rostratus*) which lacks cardenolides were completely edible to the same birds (Brower *et al.*, 1967). Subsequent work showed convincingly that the toxicity status of each individual adult monarch depends on the presence or absence of cardenolide secondary metabolites of the host plant of its larval stage (Fink & Brower, 1981; Brower, 1984; Malcolm & Brower, 1989). Brower *et al.* (1967) coined the term 'automimicry' to describe this dimorphism in edibility, since the nontoxic (automimic) individuals are assumed to gain protection from the presence of their toxic (automodel) conspecifics in a similar manner to that seen in Batesian mimicry (see also an analogous study for *Danaus chrysippus* in Brower, Edmunds & Moffitt, 1975). Automimicry is effective because predators cannot determine which host plant an individual adult used during its larval stage.

Dimorphism in the presence/absence of toxicity is not limited to the monarch butterfly; indeed the state of automimicry may be widespread across diverse taxa, life-history stages and ecological niches. For example, automimicry has been noted in other arthropods [such as millipedes (Eisner *et al.*, 1967) and beetles (Kellner & Dettner, 1995)]. Defensive responses are a likely source of automimicry in arthropods and other animals. Many insects can, for example, defend themselves by defensive secretion (by regurgitation, reflex bleeding etc.; Whitman *et al.*, 1990), and a nontrivial proportion of a prey population may be automimics in the sense that they do not produce any defensive secretion when threatened (Higginson *et al.*, 2011; Nokelainen *et al.*, 2012), either because they choose not to respond, or because they do not have defensive fluids available. There is in addition some potential for automimicry in populations of insect eggs, where a female confers toxicity on only a subsample of her ova (Hare & Eisner, 1993), leaving others undefended. A number of laboratory studies show that animals often lose their toxicity if they are reared on unnatural food plants, suggesting that dietary variation could lead to automimicry in the wild (review in Ruxton, Sherratt & Speed, 2004).

The concept of automimicry can be similarly applied to many cyanogenic plant species in which some plants or some leaves within individual plants are acyanogenic, whereas others are chemically defended and cyanogenic. Perhaps, the best known case studies of chemical automimicry in plants focus on white clover *Trifolium repens*, but many other species show automimetic variation in cyanogenesis (Till, 1987; Till-Bottraud, Kakes & Dommee, 1988; Poulton, 1990; Hughes, 1991; Till-Bottraud & Gouyon, 1992; Goodger *et al.*, 2002; Zagrobelny *et al.*, 2008). Acyanogenesis within plants may mirror the evolution of 'empty flowers' on individual plants, which fail to reward polinators (Bell, 1986; Gilbert, Haines & Dickson, 1991; Smithson & Gigord, 2003).

Furthermore, automimicry is not confined to complex eukaryotes. Some bacteria for example secrete toxic secondary metabolites which provide defence against protozoa and other predators (Jousset, 2011). In the soil bacterium *Pseudomonas fluorescens*, mutants that do not secrete defensive secondary metabolites may co-exist with secreting bacteria, such that the nonsecretors can be thought of as automimics (see data in Jousset *et al.*, 2009). As we describe below, this bacterial example is an important test of functional explanations for automimicry. Though not strictly to prevent predation, 'killer toxins' are known in yeasts. These are believed to exist because they kill sensitive yeast species, reducing competition (Pintar & Starmer, 2003), and they are notably dimorphic, with populations containing both killer and non-killer yeasts.

There are a number of evolutionary explanations to account for the persistence of automimics within prey populations, and we now consider them in turn.

(*a*) *Automimicry as parasitism on a public good*

There may be an overarching evolutionary explanation for automimicry, caused by two conflicting effects of toxins on prey that bear them. First, toxins may be costly to the prey that use them, in the sense that they decrease Darwinian fitness in the absence of antagonists such as predators. Second, toxins provide alternative lines of defence for prey, one of which can be exploited by nontoxic 'cheats'. One line of defence is individual protection from injury during attack. Many toxins, for example, are unpalatable or irritating, providing predators with an incentive to release prey with little or no damage (Wiklund & Järvi, 1982). Toxic defences of an individual however also contribute to a 'common good', by educating predators about the aversiveness of prey and hence deterring future attacks on that or other individuals of similar appearance. Nontoxic automimics may benefit by exploiting this common protection, gaining from the reduced attack rates caused by the presence of toxic conspecifics, but paying no individual cost of toxicity themselves.

Cheating as an explanation for automimicry has been evaluated in several theoretical models (Till-Bottraud & Gouyon, 1992; Guilford, 1994; Broom, Speed & Ruxton, 2005; Ruxton & Speed, 2006; Speed, Ruxton & Broom, 2006; Svennungsen & Holen, 2007; Svennungsen, Holen & Leimar, 2011). What can prevent automimics from taking over the whole population in these models is the assumption that predators adjust their attack rates as they learn about the average toxicity of the prey population, attacking more prey as automimics become common. In this way automimics parasitise and deplete the common good of predator avoidance. Though cheats may invade a population of defended prey they will lose fitness as they increase in abundance because they encourage attacks. Since automimics are likely to be ingested when attacked, they tend to lose fitness more rapidly than defended prey, which are often rejected, as attacks increase in frequency. After sufficient evolutionary time, cheats rise to a stable equilibrium frequency at which the strategy of the automimic cheat (which pays no costs of toxicity but is vulnerable to injury from attacks) has exactly the same fitness as the strategy of the automodel (which pays the costs of toxicity but is relatively more able to resist attack). This cause of automimicry is then one in which fitness declines with frequency (i.e. negative frequency dependence). Till-Bottraud & Gouyon (1992) originally represented this scenario in a model of automimicry in cyanogenic plants, in which the optimised trait was the number of leaves within a plant that are acyanogenic. Similar models were later presented in the context of automimicry in animals (Broom *et al.*, 2005; Ruxton & Speed, 2006; Speed *et al.*, 2006; Svennungsen & Holen, 2007). At equilibrium, automimics are predicted to be relatively common if the costs of defence are high or if defended 'automodel' prey are highly repellent to predators.

(*b*) *Tests of the public good hypothesis*

To verify the hypothesis that automimicry emerges as a form of parasitism on a public good, we need initially to verify three assumptions.

One key assumption is that the *per capita* rate of attacks on a prey population increases with the abundance of edible automimics. There is support for this prediction in the insect-bird literature (Brower, 1960; Brower, Pough & Meck, 1970; Pough *et al.*, 1973; Lindström, Alatalo & Mappes, 1997; Skelhorn & Rowe, 2005). A second important assumption is that defended prey have a higher probability of surviving an attack than nondefended automimics. This would apply when predators can sense the toxicity of a prey before ingestion, rejecting it without killing it (Brower, 1984). Again there is considerable support for this assumption in the insect-bird literature (Fink & Brower, 1981; Gamberale-Stille & Guilford, 2004; Skelhorn & Rowe, 2005, 2006), in cyanogenic plants (Till-Bottraud & Gouyon, 1992; Gleadow & Woodrow, 2002) and some support in the microbe literature (Jousset *et al.*, 2009).

The third necessary assumption is that chemical defences are costly. Although costs to toxicity are not always apparent (Ruxton *et al.*, 2004; Lindstedt *et al.*, 2010*a*), there is a large and growing body of literature demonstrating costs, in the form of reduced physiological efficiency, manifested in mortality or delayed growth. In the monarch butterfly for example (and in other arthropod species) the presence of toxic secondary metabolites in the animal's diet are clearly detrimental to fitness, in the sense that they reduce growth and survivorship rates compared to animals on the same diet, minus the secondary metabolites (Bowers, 1992; Berenbaum & Zangerl, 1994; Zalucki *et al.*, 2001; Ruxton *et al.*, 2004). Regurgitation, reflex bleeding and other defensive secretions which function as short-term responses to immediate predatory threats often incur the loss of body fluids and the costly replenishment of defensive chemical constituents (Whitman *et al.*, 1990). These effects are again likely to be detrimental to components of fitness such as growth rates, body size, probability of surviving to adulthood and fecundity (Rowell-Rahier & Pasteels, 1986; Grill & Moore, 1998; Higginson *et al.*, 2011). Where an animal's toxicity depends on its diet, there may be costs of locating the right foods. Cyanogenesis in plants may incur a range of costs, including increased vulnerability to frost damage, increased susceptibility to disease and reduced density of flowers (Dirzo & Harper, 1982). Hence there is in our view good evidence for the assumptions used in public good models of automimicry.

The most persuasive and exciting demonstration of automimicry as a stable outcome of frequency-dependent selection is from an experiment recently reported by Jousset *et al.* (2009) and Jousset, (2011), using the rhizosphere bacterium *Pseudomonas fluorescens*. This bacterium secretes secondary metabolites when the population is at a sufficiently high density. These metabolites presumably have a number of functions, but repelling predators such as nematodes and amoebae appears to be important. In isolation, the nontoxic

strain grew faster than the toxin-secreting strain (Jousset *et al.*, 2009), indicating that it was freed from the metabolic costs of creating and secreting secondary metabolites. In keeping with a 'parasitism of public good' interpretation, predators tended to deplete the population more effectively when the nontoxic prey was common than when it was rare. Jousset *et al.* (2009) examined predation first by nematodes, which were relatively nondiscriminating between defended secretors and nondefended nonsecretors, finding a stable automimic frequency of 26%. By contrast, predatory amoebae were considerably more discriminating, seeking out and eating nondefended individuals, and therefore forcing the automimics to a lower stable level, at 18% (see also Pough *et al.*, 1973).

This system is however, not as simple as presented in the theoretical models. Even without predators, the mutant strain shows some level of negative frequency-dependent survival, with a stable frequency of 37%. In addition, pure strains of nonsecreting bacteria have lower carrying capacities than pure strains of secreting bacteria, suggesting that the secondary metabolites have some other beneficial effects beyond deterrence of predation. Nonetheless, the experiments of Jousset *et al.* (2009) demonstrate that frequency-dependent predation by predators can help determine the equilibrium frequency of organisms that 'cheat' in relation to toxicity. Similar frequency-dependent exploitation of public good may be found in other microbial contexts (Dugatkin *et al.*, 2005; West *et al.*, 2007).

(*c*) *Automimicry in defensive secreters: public good or simple depletion?*

Animals that use up a limited resource whilst defending themselves during an attack may be unable to mount a similar defensive response until some period has elapsed when the depleted resource has been replenished. Depletion of a defensive secretion in a proportion of individuals will cause automimicry within populations, in the sense that some are able to defend themselves, and others not. Many arthropods lose nontrivial volumes of repellent substances that they secrete in order to defend themselves from attack. Examples include reflex bleeding in ladybirds, venomous stings in wasps, and regurgitation of gut contents in many arthropods (see Whitman *et al.*, 1990). In these examples the extent of automimicry is perhaps entirely caused by the recent history of attacks on individuals and may thus be explained without the need for any considerations of frequency dependence. However, if synthesis and storage of defensive secretions is itself costly, which seems often likely to be the case (Rowell-Rahier & Pasteels, 1986; Grill & Moore, 1998; Higginson *et al.*, 2011), there may still be scope for cheating and frequency-dependent explanations of automimicry, in the sense that some individuals may 'choose' not to invest in a secretion whilst conspecifics continue their investments. An example is the recent work of Daly *et al.* (2012), who examined the caterpillars of the Large White butterfly (*Pieris brassicae*), that defend themselves by regurgitation of gut contents. Daly *et al.* found that the frequency of nonregurgitating automimics increased with the local density of the caterpillars. One interpretation of this result is that the perceived *per capita* risk to individuals declined as the group size increased, hence the equilibrium frequency of automimics increased.

Animals that lose defensive fluids during defensive responses may therefore present good model systems for testing theoretical models of automimicry.

(*d*) *Environmental variation and competition*

The direct-protection *versus* public benefit explanation for the persistence of automimicry is in our view compelling, but it is of course not the only explanation. We now consider alternative causes of automimicry, starting with habitat heterogeneity. The most obvious proximate cause of automimicry in organisms that sequester their defences from their foods is heterogeneity in food supply (Brower *et al.*, 1967; Bowers, 1992), so that some foods confer toxicity and others do not. A simple explanation of 'environmental heterogeneity' may then suffice to explain the presence and abundance of automimics within a population.

However the toxicity levels of prey organisms may not merely passively reflect the heterogeneous distribution of defensive secondary metabolites in prey diets. Rather, prey may be forced to use plants that confer no toxins by intra-specific competition, in which case the proportion of automimics in the population reflects both variability in the feeding environment, and adaptive responses to the costs of competition (*c.f.* Brower, 1984).

If we consider that acquisition of toxicity depends on the availability of a limited resource, such as use of a toxin-conferring host plant, then costs of competition will be imposed when the prey population is sufficiently large compared to the host population. With strong competition, individual prey obtain fewer resources and suffer delayed growth and/or increased mortality. As competition for access to the host plant increases the net benefits of that host plant therefore decrease. A point will be reached at which competition is so strong that the use of the toxin-conferring host plant is only as useful as some alternative plant which confers no toxicity. The stable evolutionary strategy will now be for a proportion of the population to use the toxin-conferring plant, and the remainder the alternative. At evolutionary equilibrium the fitness of defended prey (toxin, but high competition) will match those of the undefended prey (no toxin, but lower competition). In this way, ideas of the so-called 'ideal free distribution' (Stephens & Krebs, 1986; Hughes, 1993) can be applied to explain stable dimorphisms in toxin contents. Evolutionarily stable automimicry is again explained by a cost (competition) and by frequency dependence. Here though, frequency dependence is caused by the cost itself, by intraspecific competition, rather than through parasitism of a public good. Applying ideas of ideal free distributions, we note that we could explain automimicry without requirements that (*i*) toxins themselves are costly or (*ii*) predators adjust attack rates according to the frequency of automimics, or (*iii*) that they reject defended prey more often than undefended prey whist attacking them.

(*e*) *Variation in age may explain some examples of automimicry*

Individuals of particular age classes or a particular sex may be more or less likely to be automimics. In the monarch butterfly for example, it has been demonstrated that the concentration of active cardenolides declines with age (Alonso-Mejia & Brower, 1994). Individuals that fed as larvae on host plants that confer high levels of cardenolides (*Asclepias humistrata*) are likely to remain inedible to their predators throughout their lifetimes, even though they become less toxic with age. By contrast, those that obtain lower levels of cardenolide (from *Asclepias syriaca*) may lose sufficient aversiveness that they become palatable as they age (Alonso-Mejia & Brower, 1994). Age effects are clearly more complex than a simple presence/absence of toxicity, but there is a sense in which we can consider it automimicry if it renders a proportion of the population effectively nondefended. Alonso-Mejia & Brower (1994) suggest a number of proximate causes for these changes including excretion and denaturation of the cardiac glycosides, binding to the cuticle, and physical loss of body parts such as scales. A decline in toxicity may not be in any way selectively advantageous to the butterflies, and this could be a nonadaptive explanation for automimicry. However, a decrease in toxin load over ontogeny could be open to an adaptive explanation if the relative benefits of (still costly) toxins decline with age as risk of death from other factors unrelated to predation increases. In such circumstances it may be optimal to reduce investment in anti-predatory investment, if this allows a reduction in the recurrent costs of maintaining the toxins (or indeed if the toxins can be used as an energy source).

(*f*) *Toxicity is conferred unevenly by bacterial endosymbionts*

In the rove beetle *Paederus riparius,* the defensive haemolymph toxin peridin is passed across generations by maternal transmission. Toxicity is apparently caused by a form of *Pseudomonas* bacterial symbiont and horizontal transmission can occur when adults cannibalize infected eggs (Kellner & Dettner, 1995, 1996; Kellner, 2001, 2002). Females that lack the symbiont fail to produce the toxin. In this case, the proximate mechanism of automimicry is based on the presence or absence of infection, and is best explained by epidemiological modelling, none of which has been applied to the question of automimicry to our knowledge. It is thus not clear whether the persistence of automimics is caused in part by simple frequency dependence or by other more complex epidemiological factors.

(*g*) *Spatiotemporal variation in predation risk*

Besides frequency dependence, another common explanation for stable dimorphisms (and indeed stable polymorphisms) is heterogeneous selection. In this way, within-population variation in investment in defence may arise because there are some habitats and/or some generations when investment in defence is selected and some when such investment is not (Ruxton & Speed, 2006). If there is a heritable contribution to the quantity that an individual invests in toxicity (see data and discussions in Eggenberger & Rowell-Rahier, 1992; Müller *et al.*, 2003; Yezerski, Gilmor & Stevens, 2004), then temporal variation in selection might be expected to lead to within-population variation at any given moment in time. Similarly if there is mixing of individuals among habitats with different selection pressures, then again this may lead to among-individual variation in investment at a particular point in space. Another means of generating heterogeneous selection is when the prey are attacked by different predators or enemies in different locations, or even in the same location (Gibson, 1984; Losey *et al.*, 1997; Endler & Mappes, 2004).

(2) Widespread quantitative variation in toxins

The simple dichotomy of defended/undefended prey seen in automimicry is of course a special case of wider variation in the quantity of toxins. In the following sections we focus on explanations that best apply to more widespread quantitative variation. Note that the importance of competition in causing widespread quantitative diversity will be considered later (Section III.2) alongside explanations for qualitative diversity.

(*a*) *Stochastic environmental effects versus individual control over toxicity levels*

An obvious explanation for widespread variation is that it reflects the stochastic nature of the environmental components that influence toxin levels. As has been noted elsewhere (Blum, 1981; Bowers, 1992), a large number of factors may affect the precise level of toxicity recorded within an individual: age (see Section II.1*e*), body size, sex, and availability of raw materials for biosynthesis or sequestration. Thus it is perhaps not surprising that toxicity is often so variable within populations. However, environmental variation may not be the whole story, particularly if prey can control their own toxicity levels in the face of environmental heterogeneity. Some animals that directly sequester defensive chemicals from their diets exert considerable control over the levels of toxins that they sequester. Malcolm & Brower (1989) examined the relationship between the levels of sequestered toxins within monarch butterfly larvae and those found in 12 of their milkweed host-plant species (data from a series of papers starting with Brower *et al.*, 1982). These host plants varied widely in their concentrations of cardenolide secondary metabolites (measured as μg equivalent to digitoxin 0.1 g dry mass, they ranged from 886 for *A. asperula* to 17 for *A. fascicularis*). Toxin measures in the animals did not however always vary with toxin measures in the plants. Whereas there was a good match of animals to their plants for intermediate toxin values (Fig. 1: *circa* 300–350 μg), animals fed on plants with low toxin levels had higher toxin levels than their plants whereas those fed on plants with high toxin levels had lower toxin levels than their plants (Fig. 1). Malcolm & Brower (1989) argue that their data indicate that the monarch is adapted to control individual levels of chemical defence and to regulate those levels within

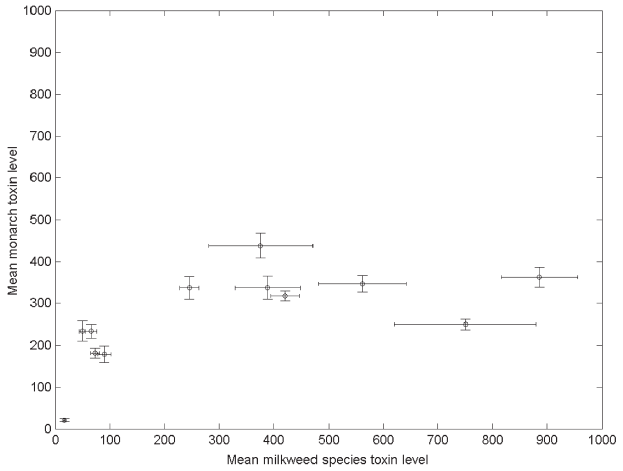

Fig. 1. Scatterplot of data for cardenolide toxin concentrations in milkweed (*Asclepias* spp.) host plants and monarch butterflies (*Danaus plexippus*). Drawn from data from Table 1 in Malcolm & Brower (1989), units are μg equivalent to digitoxin 0.1 g dry mass). Error bars are +/-2S.E.M.

a relatively narrow range of tolerance. Furthermore such control of individual toxicity is not limited to the monarch: a similarly active regulation to a 'target' level of toxin was recently reported in the sequestration of iridoid glycoside secondary metabolites by larvae of the generalist aposematic herbivore *Parasemia plantaginis* (Arctiidae) (Lindstedt *et al.*, 2010*b*). So while it is true that environmental variation causes variation in toxicity levels, the monarch and *P. plantaginis* larvae are not necessarily mirroring external sources of variation. In the monarch, this is especially true when the host plant stores toxins at much higher levels than the caterpillars usually sequester. If there are costs to toxicity it is easy to construct models in which ever higher levels of toxins are not always beneficial and there is a single optimal value for toxicity (Speed & Ruxton, 2007); one could interpret these data in that context. Variable factors such as costs of toxin storage then explain variation in toxin levels, rather than environmental variation itself.

(*b*) *Absence of, or weak selection over a large phenotypic range*

One way to explain persistent quantitative variation in toxin levels within a species would be to assume that toxins are cost free, and that their deterrent effects saturate after some threshold. Phenotypes below this threshold will be less effective at deterring predation than those above it, and will tend to be removed from the population. However, above the threshold phenotypes would be selectively neutral, equally good at preventing predation and injury from attack and hence subject to the forces of mutation and drift. Very weak selection could produce similar levels of diversity to no selection at all, so that if costs of toxicity were present but very small, toxin variation may persist either through very slow directional selection or *via* mutation-selection balance.

Given the strong emphasis on the costliness of toxicity in the preceding sections, an absence of costs might seem unlikely as an explanation for toxin diversity. It should be pointed out however, that there are instances in which chemical defence has either no demonstrable costs (Lindstedt *et al.*, 2010*a*) or where the individual animal makes energetically profitable molecules as a byproduct of toxicity (Rowell-Rahier & Pasteels, 1986; Ruxton *et al.*, 2004). Furthermore it may be that for many specialist herbivores, chemicals are cost-free at some stages of the life history but not others. In the Glanville fritillary (*Melitaea cinxia*), for example, diets with iridoid glycosides are detrimental to first-instar larvae, but beneficial to the growth and development of later instars (Saastamoinen *et al.*, 2007). It may be possible to argue that organisms that sequester secondary defences in may make use of existing physiological mechanisms for dealing with dietary toxins, making the defence itself cheap. However Dobler's (2001) review of the special adaptations necessary to enable effective sequestration in herbivorous insects suggests that there are a number of specific proximate costs to sequestration. These adaptations include transport across the gut wall (perhaps *via* a carrier molecule), transport to the point of storage, and prevention of autotoxicity (for example, by changes to the amino acid sequences of enzymes, or the creation of glandular storage devices, see also Bowers, 1992). In summary, variation in toxin levels can be explained if costs can be demonstrated to be negligible, and if the efficacy of a toxin rapidly saturates. This possibility awaits application to systems in which toxins are known to be actually or almost cost free.

III. VARIATION IN THE CHEMICAL PROFILE OF CHEMICAL DEFENCES

In this section we consider the most complex case: variation in the biochemical profile of different defensive chemicals among individual animals within populations. The profile may vary in the number of different molecules present and/or in their relative or absolute abundances. Biochemical profiles of defences often uncover a complex cocktail of molecules, with numerous minor constituents found at low levels (Pasteels *et al.*, 1983). Some of the explanations for variability described in the previous section will apply here too, and hence we focus on those not already considered.

(1) Variation in biochemical profiles are of no adaptive significance

There are at least two reasons to believe that variation in chemical profile could sometimes have no effect on the survival of the individual within a population. First, chemical complexity of many secretions, especially in organisms that biosynthesise their defensive toxins, may be explained by the variable presence of biosynthetic enzymes, and enzyme precursors remain in the defensive fluid after catalysis is complete. Pasteels *et al.* (1983) argued that variation in such protein components may be widespread within populations, but have no significance for the efficacy of the defence itself.

A second explanation, similar to that in Section II.2*b* is that there may be considerable functional redundancy within a secretion, so that variation in the precise biochemical profile may be neutral with respect to the efficacy of the defence (Tschinkel, 1975; Pasteels *et al.*, 1983). If the costs and efficacy of alternative profiles are very similar, then widespread diversity within a prey population is easy to predict. This explanation was proposed by Jones *et al.* (1986) to explain the very widespread variation in chemical profiles seen within the lubber grasshopper (*Romalea microptera*).

An interesting potential example of redundancy is seen in insects that have two forms of defence: one sequestered, the other biosynthesized. If both forms of defence are costly and habitats are heterogeneous, so that sequestration may be better in some microhabitats and biosynthesis in others, we could reasonably imagine that the same net benefit to the animals could accrue with alternative chemical profiles, and hence chemical profile variation could be stable (Pasteels *et al.*, 1995).

(2) Competition

One explanation described above for automimicry in animals that rely on some dietary components for their toxicity is that the costs of competition may drive animals to use otherwise suboptimal resources, with the consequence that some individuals have no toxicity, and others are toxic (see Section II.1*d*). It is possible to explain variation both in quantity and chemical constituents of toxins by extension of this idea. We assume that a prey is polyphagous, with foods ranked in proximity to the optimal chemical toxin profile. Increasing competition for preferred foods decreases their value and makes low-ranked foods relatively attractive. At equilibrium, prey on low-ranked plants would benefit from lower costs of competition (e.g. high growth rates), but develop poorer defences; prey using higher ranked plants would have higher costs of competition (so, for example lower growth rates), but would have better secondary defences. Prey could spread themselves around a potentially large number of host plants, and consequently acquire highly variable chemical profiles, but overall have the same fitness values (in essence this is again an application of the 'ideal free' distribution to explain toxin diversity, but on a larger scale). This argument is attractive because large-scale variation in toxicity can have a conceptually simple adaptive explanation, as a stable outcome of intra-specific selection.

(3) Effects of age, sex, season and mating status

As we described in Section II.1*e*, chemicals sequestered by immature forms of a prey animal may be lost as the adult form ages. If, for example, the defensive chemical degrades over time (or is lost for some other reason) the adult may have no source by which to replenish it. This need not be the case, however, for animals that continue to sequester toxins as adults or have some mechanism for their biosynthesis; in which case the effects of age may run in the opposite direction. In the brightly coloured rove beetle (*Paederus riparius*) for example, Kellner & Dettner (1995) reported a decrease in mass of total toxins stored (and in terms of concentration, μg/mg fresh mass) between larval and pupal forms, but found that females can regenerate and increase quantities of toxins as they mature, perhaps in preparation for donating the toxin to their eggs. Hence age effects are constrained to decline if toxins are only collected or synthesized during an early life stage; where life history allows collection over several life stages effects of age may be nonmonotonic, reversing in direction across the lifespan of the animal.

The chrysomelid beetle *Oriena gloriosa* has many chemical components of its defensive secretions: 'tyrosine betaine, ethanolamine, and *de novo* synthesized cardenolides' (Eggenberger & Rowell-Rahier, 1993, p. 751). Eggenberger & Rowell-Rahier (1993) examined age-related changes to 16 components of the biochemical profile of the defensive secretion in laboratory-reared individuals. The quantity and concentration of most components increased between two and ten weeks from emergence as adults, a result attributed to constraints on the rates of biosynthesis of the toxins, rather than being adaptive variation *per se* (and even here the picture is not simple, because a minority of secretions declined significantly in toxin levels as time passed). In addition to effects of age since eclosion, changes in secretions tended to differ between males and females, with females generally having larger quantities and higher concentrations of defensive chemicals than males. Furthermore mating significantly diminished the quantity of one component (RT9, see Table 1 in Eggenberger & Rowell-Rahier, 1993), and increased the concentration of others in females, suggesting that the substances may be used as pheromones in addition to chemical defence.

The effects of age on toxin variation in wild populations were illustrated when Eggenberger *et al.* (1992) compared the chemical profile of wild-caught animals in early summer to those in autumn. In autumn, members of the population were typically young, having been born over the recent summer. These animals had lower mean concentrations and lower quantities of toxins than those sampled in June, which were older, having hibernated through the previous winter. Hence breeding ecology and local seasonality interact to cause predictable changes in age structure, and hence predictable changes in average levels of components of the chemical defence of the population. The ultimate explanations for toxin variation here depend on the life-history pattern of the species itself.

Age relates directly to developmental stage, which in turn may determine the biosynthesis or secretion of toxins (see Chapter 15 in Blum, 1981), so that insect toxins may only be secreted when specific instars are reached, and even here there may be some developmental delay.

(4) Uses of defensive substances for communication

Many chemically defended insects utilize and deplete their defensive chemicals for the purposes of intra-specific communication (reviewed in Pasteels *et al.*, 1983). Defensive chemicals may be used in a derived form as mating pheromones, as for example in the pyrolizidine alkaloids (PA) of the arctiid moths (e.g. Rothschild *et al.*, 1979;

Weller, Jacobson & Conner, 1999). Males of the arctiid moth *Utetheisa ornatrix* pass quantities of PA as a nuptial gift (Dussourd *et al.*, 1991). Conner *et al.* (1981) speculated that the female response to the release of a pheromone chemically derived from defensive PAs functions as a component of mate choice to assess the defensive quality of the male's gift. Pheromones using defensive substances (or derivatives thereof) may also function in socially rather than sexually based communication, acting as alarm pheromones, or triggers of aggregation (Pasteels *et al.*, 1995). Hence, even if selection tended to favour uniformity in chemical defences, depletion by deployment in communication would tend to cause variation in the chemical profiles of chemical defences within populations.

(5) Variation in predator/enemy profiles

One reason for the complexity of defensive substances is when the various components operate against several or many different enemies. Whilst defence against predators is perhaps the function most often associated with defensive secretions, it may be that defensive substances have additional benefits such as deterring parasitoids (Sime, 2002) and microbial parasites such as bacteria and yeasts (Gross *et al.*, 1998, 2008; Gross, Podsiadlowski & Hilker, 2002).

Variation in chemical profiles could be explained if there are constraints on the effectiveness of chemical defences, preventing maximum deterrence of all possible enemies. In this scenario the individual can protect themselves well from some kinds of enemy but not others. One conceivable outcome is that alternative chemical profiles target different enemies, but provide similar overall levels of protection from predation, making them selectively neutral.

An alternative evolutionary explanation for diversity of chemical profiles would be if there are diverse enemies in relatively unpredictable abundances (see also Section: II.1*g*). Rather like the lottery ticket argument for sex (see Williams, 1975), having offspring with varied defensive phenotypes better matches an environment with an unpredictable set of enemies than does one with monomorphic defensive traits.

(6) Preferences for the familiar favour rare prey forms

If predators follow a 'better the devil you know' approach to diet choice, this may provide protection to rare prey forms, which are likely to be treated as unfamiliar by predators. Selection that favours rarity (negative frequency-dependent selection, also known as apostatic selection; Clarke, 1962), is known to be capable of maintaining diversity. Two experiments give particularly clear support to the idea that predators may prefer chemically defended prey forms that are most familiar to them, suggesting that diversity in chemically defended profiles is maintained by frequency-dependent selection.

First, Pasteels & Gregoire (1984) examined selective predation on chrysomelid beetle larvae by adult sawflies (*Tenthredo olivacea*). A key finding was that the sawfly predators developed strong preferences for familiar prey (either *Phratora vitellinae* or *Plagiodera versicolora*), and avoided the unfamiliar one. In a related experiment with domestic chicks (*Gallus gallus domesticus*) as model predators and two kinds of bitter-tasting artificial prey (Bitrex or quinine), Skelhorn & Rowe (2005) found that their model predators were much more likely to taste-reject the rarer of the two prey forms and conversely were more likely to ingest the more common form. For example, when 25% of available prey were Bitrex-flavoured and 75% were quinine-flavoured, about 80% of the Bitrex prey were taste-rejected, whereas 50% of the quinine prey were taste-rejected. These are promising results, but clearly much more work is needed to determine whether frequency-dependent selection is a cause of diversity in the chemical profiles of prey in the wild.

A related explanation for within-species diversity is that it prevents counter-adaptation by predators (Pasteels *et al.*, 1983). Counter-adaptation could occur within or between prey generations due to changes in gene expression allowing predators to tolerate better the defensive toxin in a prey. Counter-adaptation would provide a proximate mechanism by which predators come to favour abundant prey forms.

IV. CONCLUSIONS

(1) Variation in defensive toxins is widespread and likely to be ecologically significant.

(2) A key question is whether this variation - be it presence/absence (auto-mimicry), or variation in quantities and profiles of toxins - represents 'ecological noise'; variation caused by the stochastic nature of prey environments, and of no adaptive evolutionary significance *per se*.

(3) One evolutionary explanation of such 'noise' arises when prey obtain a similar level of protection across a range of toxin levels or profiles, while these profiles impose similar costs. Here we can see a good general case for toxin variation itself having no evolutionary or ecological significance. To our knowledge, there are surprisingly few datasets with which we can evaluate this prediction.

(4) Good cases can be made that toxin variation is of adaptive significance. It is easy to generate plausible accounts of frequency-dependent selection that would encourage and stabilise diversity in a population. For example, inclusion of production or storage costs can to lead to cheating and 'automimicry' in defended species: 'cheats' that do not produce toxins may benefit through the exploitation of the public good provided by prey that are toxic.

(5) Where there is sufficient cost from within-population competition for a toxin-conferring host plant, a proportion of prey may compensate by moving to food source(s) that provide no (or lower levels of) toxins, stabilizing automimicry or even very widespread variation in toxin profiles within the population. Frequency dependence may be imposed too by predator preferences, where predators favour familiar toxins over unfamilar ones.

(6) There may in addition be life-history-related explanations for toxin diversity, such as effects of age and maturity and competitive depletion of molecules in other roles such as communication. Even where dietary sources of toxins are highly variable, some prey species may control the levels of toxins that they sequester, so that observed toxin variation in a prey population does not simply mirror environmental variation.

(7) The evolutionary explanation of toxin variation has, in our view, received too little attention; we hope that this review stimulate interest and further empirical work to evaluate some of the hypotheses described herein.

V. ACKNOWLEDGEMENTS

We thank Lincoln Brower and an anonymous referee for very helpful comments on this review. MS was supported by NERC grant NE/E018521/1.

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(*Received 1 August 2011; revised 14 March 2012; accepted 19 March 2012; published online 30 April 2012*)