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Variation in chemical defences of plants may improve the effectiveness of defence

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ABSTRACT

Chemical defences of plants exhibit great variation, but their precise patterns and effects on herbivores have not been examined, especially at small scales. Recent work has demonstrated very high spatial variation at small scales within leaves. Empirical tests of the effects of variation on herbivore fitness and behaviour are therefore particularly difficult. Here I present a series of dynamic-programming models of the effects of variation in chemical defences (glucosinolates) at several scales. All models include the same mean concentration of glucosinolates. They differ only in the presence or absence of variation at each of five scales: among plants, within plants due to induction of defences in response to herbivore feeding, among leaves on a plant, among parts of a leaf, and within leaf parts. I used empirical data from *Raphanus sativus* (Brassicaceae) and the generalist caterpillar *Trichoplusia ni* (Lepidoptera: Noctuidae) to parameterize the models. Model caterpillars behave to maximize their size at pupation by choosing each day either to feed in place or to move within a leaf, between leaves or between plants. Variation among plants and within plants due to induction dramatically decreased pupation rates. The smallest scale of variation – within leaf parts – also reduced fitness by slowing growth and delaying pupation. However, small amounts of this stochastic small-scale variation slightly increased pupation rates. The results suggest that variation in plant chemical defences has important effects on fitness and behaviour of herbivores.

Keywords: dynamic programming, glucosinolates, *Raphanus sativus*, variation.

INTRODUCTION

Concentrations of chemical defence compounds in plants vary widely at many spatial and temporal scales (see, for example, Denno and McClure, 1983). Genetic variation, microsite differences and prior herbivory can cause differences among plants. Leaf age and vascular architecture can cause chemical variation among leaves or even within leaves. Patterns of

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variation have been reasonably well documented at larger scales (e.g. between plants or between parts of a plant). Those at smaller scales (e.g. within individual leaves) have not (but see Whitham, 1983; Niemalä *et al.*, 1984; Stout *et al.*, 1996; Orians *et al.*, 2000). In a previous study I found extreme variation in the concentrations of chemical defences at these very small scales. I examined small (1–3 cm^2) samples from all parts of leaves of *Raphanus sativus* (Brassicaceae) and found no correlation among glucosinolate concentrations in adjacent samples. In addition, variation at this small scale accounted for 57% of all variation in glucosinolate concentration across leaf age, plant and damage treatment (Shelton, 2002).

Several hypotheses suggest that consumption of defensive compounds in variable doses affects herbivores differently than consumption of the same amount in constant doses. Stockhoff (1993) demonstrated such an effect with nutrients rather than defences. He found that caterpillars fed a fixed amount of nitrogen in variable doses had lower fitness than caterpillars fed the same amount in constant doses. Also, some insects produce inducible detoxification enzymes in proportion to the amount of toxins consumed (Snyder *et al.*, 1993; Glendinning and Slansky, 1995; Feyereisen, 1999). The levels of these enzymes in an insect feeding on a highly variable toxin source may often be out of phase with current toxin consumption. Finally, some chemical defences have non-linear dosage-dependent effects on herbivores, such that they have little toxic effect at low or moderate doses, but are increasingly toxic at high doses (Parr and Thurston, 1972; Elliger *et al.*, 1976; Byers *et al.*, 1977). A herbivore that consumes a single high dose, rather than consuming the same quantity in several smaller doses, should be more negatively affected (Karban *et al.*, 1997; Shelton, 2000).

We know little about how this variation actually affects the fitness and behaviour of herbivores. To determine whether distribution of chemical defences alters the effectiveness of plant defence, we must know how the pattern of distribution affects herbivores. Second, we must know how patterns of chemical variation at different scales in a plant or population of plants interact to affect herbivores. Addressing these questions empirically is difficult. The high variation in concentrations of chemical defences in plants, especially at small scales, makes it difficult to know precisely what concentration of defensive compounds herbivores consume. Concentrations could be controlled in experiments using artificial diets. But the many synergistic effects between other aspects of plant chemistry, such as water content, nutrient level and pH, make applicability of such studies to herbivores feeding on live plants questionable. Computational models may be better able to address these questions. In particular, a model parameterized with empirical data about patterns of chemical variation at multiple scales in plants, and how these chemicals affect herbivore fitness and behaviour, can reveal how herbivores should respond and how they are affected.

I developed a series of dynamic-programming models to explore the effects of chemical variation at multiple scales, corresponding to the scales of variation in glucosinolate concentrations found in *Raphanus sativus* plants (Shelton, 2002). The breakdown products of glucosinolates (e.g. isothiocyanates) are mildly toxic to a wide variety of generalist herbivores (Blau *et al.*, 1978; Louda and Mole, 1991; Agrawal, 2000) and to some specialist herbivores (Agrawal and Kurashige, 2003). I focused the models on the effects of glucosinolates on a generalist caterpillar, *Trichoplusia ni* (Lepidoptera: Noctuidae), using data on their growth and behaviour from laboratory experiments in which they were fed on leaves of *R. sativus* that varied in glucosinolate concentration.

Table 1. Summary of the scales of chemical variation included in each model

Model	Among plants	Induction	Among leaves	Between leaf parts	Within leaf parts
A	–	–	–	–	–
B	+	–	–	–	–
C	+	+	–	–	–
D	+	+	+	–	–
E	+	+	+	+	–
F	+	+	+	+	+

Note: Each scale of variation was based on empirical data of glucosinolate concentrations in *Raphanus sativus*.

I examined five scales of chemical variation using six related models. The first model (model A) includes no variation in glucosinolate concentration – the concentration was the same in all parts of all plants. Each successive model (Table 1) adds variation in glucosinolate concentration at a smaller scale: among plants (model B), within plants due to induction of defences in response to herbivore feeding (model C), between two leaves on a plant (model D), between lower and upper halves of leaves (model E), and among random areas within leaf halves (model F). The distribution of glucosinolates in each model was determined probabilistically from empirical data on glucosinolate concentrations in *R. sativus* (Shelton, 2002). Each model predicts the behaviour and expected fitness of an optimally behaving caterpillar in a population of plants with the specified levels of chemical variation.

The primary purpose of these models is to examine how spatial variation in glucosinolate concentrations, as opposed to differences in mean concentrations within and among plants, affects the behaviour and fitness of herbivores. And, if spatial variation of defences is important to herbivores, what scales of variation have the greatest effects?

THE MODELS

All the models operate in the same way, differing only in the variation in glucosinolate concentrations. The models follow a caterpillar feeding in a population of plants. Caterpillars are characterized by their weight, x, and plants are characterized by the glucosinolate concentration where the caterpillar is currently located. This concentration is a function of the initial glucosinolate level, s_0, and the amount of feeding, q, the caterpillar has done, which determines the degree of induction of the plant's defences.

Caterpillars can feed in place or move one of three 'distances' – between plants, between leaves or within leaves. I assume caterpillars can perceive the glucosinolate concentration where they are feeding and the distribution of glucosinolate concentrations in the population of plants, but cannot perceive the glucosinolate concentration they will encounter if they move. The caterpillar selects the behaviour that leads to the maximum expected size at pupation, which is correlated with fecundity in most Lepidoptera (e.g. Courtney, 1981). The functions used in the models and their parameterization from data are described below. Table 2 lists the parameter values used.

Table 2. Summary of variables and values used in the model

Parameter	Definition	Value used
	State variables	
x	weight of caterpillar (mg/10)	0–25[a]
s_0	initial toxin level of plant before induction (4 μmol glucosinolate per cm^2 leaf)	0–15[a]
q	amount of feeding damage to plant (cm^2)	0–20[a]
t	time (12-h steps)	1–60[a]
	Caterpillar parameters	
x_{pup}	minimum weight for pupation	10[a]
k	consumption rate proportional to caterpillar weight	0.2613[a]
A	assimilation rate of food dependent on consumption	1.0865[a]
C_{ratio}	proportion of energy from food used for basal metabolism (defined at $x = 10$)	0.1[b]
D_{ratio}	proportion of energy from food used for detoxification (defined at $s = 2$)	0.05[b]
α	metabolic cost as proportion of body weight to the 3/4 power	0.0594[c]
β	total energy gained per unit of food	1.2782[c]
γ	detoxification cost per unit of toxin	0.0160[c]
v	degree of non-linearity in detoxification costs	2.0[b]
μ_{max}	maximum movement cost	6[b]
a_{WL}	cost of moving within a leaf as proportion of cost of moving between plants	0.2[b]
a_{WP}	cost of moving between leaves as proportion of cost of moving between plants	0.3[b]
M	exponential mortality rate	0.01[b]
	Plant parameters	
r	induction rate	0.2716[b]
ρ	probability that the toxin level of leaf will change during feeding as a result of within-leaf variability	0.3[b]
s_{low}	glucosinolate concentrations in low category	$0 < s \leq 5$[a]
s_{med}	glucosinolate concentrations in medium category	$5 < s \leq 10$[a]
s_{high}	glucosinolate concentrations in high category	$10 < s \leq 15$[a]
$\bar{s}_{low}$	mean glucosinolate concentration for low category	2.4549[a]
$\bar{s}_{med}$	mean glucosinolate concentration for medium category	6.9631[a]
$\bar{s}_{high}$	mean glucosinolate concentration for high category	12.6199[a]
	Environmental parameters	
Pr(damaged)	proportion of previously damaged plants in the environment	0.5[b]
Pr(young)	proportion of young leaves on a plant	0.5[b]
Pr(leaflet)	proportion of a leaf that is lower leaflets	0.5[b]

[a] Values estimated from empirical data on *Raphanus sativus* and *Trichoplusia ni*.
[b] Values are best estimates determined by examination of model output over a range of values.
[c] Values determined from empirical values for k and A, with the estimates for C_{ratio} and D_{ratio}.

Caterpillar growth

Caterpillar growth is a function of weight gain from feeding and weight loss from metabolic, detoxification and movement costs. I estimated the parameters for weight gain and consumption for *Trichoplusia ni* caterpillars from laboratory experiments. Because movement was restricted in these experiments, I assumed movement costs were negligible and defined weight gain as

$$X(t+1) - X(t) = B(x) - C(x) - D(x,s) \tag{1}$$

where $B(x)$ is total weight gained from food, $C(x)$ is metabolic cost, and $D(x,s)$ represents detoxification costs. In experiments, consumption increased in proportion to weight, and weight increased in proportion to consumption (Shelton, 2002), so

$$B(x) = \beta k x \tag{2}$$

where k is the consumption rate estimated from experiments and β is analogous to the assimilation rate.

Metabolic costs increase in proportion to the 3/4 power of weight for many organisms (Stahl, 1962; West *et al.*, 1997). I therefore set

$$C(x) = \alpha x^{3/4} \tag{3}$$

Detoxification costs depend on the amount of toxin consumed, which is the product of the concentration of toxin, s, and the leaf area consumed, kx:

$$D(x,s) = \gamma s^{v} k x \tag{4}$$

The parameter v represents the degree of non-linearity of increasing detoxification costs. If $v = 1$, detoxification costs increase linearly with glucosinolate concentration. If $v > 1$, higher concentrations are more costly to detoxify. Ample experimental evidence (e.g. Blau *et al.*, 1978; Louda and Rodman, 1983; Chew, 1988; Wolfson, 1991) suggests that for glucosinolates $v \geq 1$ because small doses generally have little effect on herbivores but increasing doses have increasingly strong effects. I varied the value of v to explore its effects on model predictions.

The relative magnitudes of metabolic costs, detoxification costs and energy gain from food are unknown, so I assumed values for the proportion of the total energy from food used for metabolic, C_{ratio}, and detoxification costs, D_{ratio}. I varied these values to explore their effects on the predictions of the models. I combined equations 1–4 and used the empirically estimated slope for growth rate per unit consumption, A, to solve for α, β and γ at $x = 10$ (intermediate caterpillar weight) and $s = 2$ (mean glucosinolate level in an undamaged plant). These values are listed in Table 2.

To estimate costs for the three movement distances, I assumed a baseline cost for movement between plants (BP). I then set costs for between-leaf (BL) and within-leaf (WL) movement equal to proportions of that cost. I made movement more costly for small caterpillars than for large caterpillars by making costs proportional to the length of the caterpillar, $x^{1/3}$. The cost of moving between plants is

$$\mu_{\text{BP}} = \mu_{\max} x^{-1/3} \tag{5}$$

μ_{BL} and μ_{WL} are equal to this function multiplied by a_{BL} and a_{WL} (Table 2).

Distribution of glucosinolate concentrations

Most plants in the Brassicaceae, including *Raphanus sativus*, respond to herbivore feeding by systemically increasing glucosinolate concentrations (Koritsas *et al.*, 1991; Bodnaryk, 1992; Agrawal *et al.*, 1999). The increase is approximated by a linear regression of glucosinolate concentration on amount of leaf consumed (Shelton, 2002). I modelled the induced toxin level, s, as

$$s = s_0 + rq \tag{6}$$

where r is the slope of a linear regression of glucosinolate concentration on amount of leaf tissue consumed, q, and s_0 is the initial concentration.

To determine the frequency of different glucosinolate concentrations in each model, I used empirical data from *R. sativus* (Shelton, 2002). The distribution of glucosinolate concentrations in *R. sativus* is approximately log-normal, but a log-normal distribution underestimates the probability of high glucosinolate concentrations. High concentrations may have particularly strong effects on herbivore fitness and behaviour (Shelton, 2000). Therefore, I used probability estimates derived directly from the data rather than assuming a distribution. To simplify these calculations, and to increase the accuracy of estimating probabilities of different concentrations, I divided the range of observed glucosinolate concentrations into three categories: low, medium and high. Average glucosinolate concentrations varied among parts of a plant and among plants. Damaged plants, young leaves and the lower halves of leaves had significantly higher glucosinolate concentrations than undamaged plants, older leaves and the upper halves of leaves (Shelton, 2002). I used these traits to define eight leaf types, and set the proportion of induced plants, young leaves and lower parts of leaves as parameters to determine the frequency of each leaf type (Table 2). The probability of each glucosinolate category was determined from the empirical data according to the scales of variation included in the model. I constrained the average glucosinolate concentration in a plant to be the same in all models, so that the models differed only in the scales of variation included. In addition, I examined only the presence or absence of variation at each scale. The magnitudes of variation were those observed empirically. The details of these calculations are described in the Appendix.

The glucosinolate concentration a caterpillar encounters after a move depends on the concentration where it was before the move and the distance it moved. I assume caterpillars can determine the concentration of glucosinolates only by direct contact (Louda and Mole, 1991; Fahey *et al.*, 2001). They cannot intentionally move to a lower concentration of glucosinolates. Because I assume they know the overall distribution of glucosinolate concentrations, they can move to a region with a greater probability of lower glucosinolate concentration. Although glucosinolates do differ among leaves of different ages (Porter *et al.*, 1991; A.L. Shelton, unpublished data), I assume for simplicity that the glucosinolate concentration of a leaf does not change due to ageing of the leaf while a caterpillar feeds on a leaf. Caterpillars therefore experience the differences between young and old leaves as spatial variation as they move within a plant, rather than as temporal variation within a leaf.

The new glucosinolate concentration is calculated in a two-step process. First, because caterpillars know the current glucosinolate concentration but not precisely where they are on the plant, I calculated the probability that the caterpillar is on each of the eight leaf types (defined by induction, leaf age and leaf part), given the current glucosinolate concentration. I then determined the probability of each glucosinolate concentration given the distance

moved (BP, BL or WL). The movement distance determines the leaf types the caterpillar can encounter. For example, a caterpillar on a damaged plant that moves within a leaf cannot encounter concentrations characteristic of an undamaged plant. Whenever a new plant is encountered, its initial glucosinolate concentration is selected from the distribution of glucosinolate concentrations for that model with $q = 0$.

In model A, glucosinolate concentrations are constant. In models B–E, concentrations change only when the caterpillar moves. In model F, the glucosinolate concentration where the caterpillar is feeding may change with a probability, ρ, to reflect small-scale stochastic variation within leaves. Such variation is extreme in *R. sativus* (Shelton, 2002). If the glucosinolate concentration does change because of this spatial variation, the new toxin level is determined as if the caterpillar had moved within the leaf. Because the actual frequency with which caterpillars encounter small-scale variation is unknown, I varied the value of ρ from 0 to 1 to explore its effects on model predictions. The details of these calculations are described in the Appendix.

Model computation

For each model, I calculated (by backward solution; Clark and Mangel, 2000) a set of decision rules conditioned on the caterpillar's size and the glucosinolate level and degree of damage to the plant. I then conducted simulations (forward solution) to generate predictions of caterpillar behaviour, growth and fitness that I could compare with empirical data. In each 12-h time step, a caterpillar can feed, move within a leaf, move between leaves, move between plants or pupate. To move, a caterpillar must be large enough to afford the movement cost, and in order to pupate, it must be at least a minimum weight, x_{pupation}.

When a caterpillar pupates, its fitness is determined as its expected egg load, which is a function of its weight, as determined from data on *Pieris rapae* (Lepidoptera: Pieridae) (Courtney, 1981), multiplied by a time-discounting function that rewards earlier pupation.

$$\phi(x,t) = \begin{cases} (1.58kx - 57.3)(-0.167t + 1.5) & \text{if } x \geq x_{\text{pupation}} \\ 0 & \text{otherwise} \end{cases} \tag{7}$$

Early pupation can be advantageous because offspring may experience less competition for resources and there is a greater possibility for additional broods in the season. This fitness function is also the end condition used in the final time step, equivalent to the end of the season, when caterpillars must either pupate or die.

The fitness value of feeding depends on caterpillar weight, glucosinolate concentration and time in the season. It is the expected fitness of the caterpillar, discounted for the risk of mortality, M, and in model F, averaged over the probability, ρ, that the glucosinolate concentration will change because of spatial variation within the leaf. The weight of the caterpillar increases with input of energy from food and decreases with loss to metabolic and detoxification costs. If the glucosinolate concentration changes because of spatial variation, the new concentration is selected as if the caterpillar moved within a plant, but consumption, q, still increases. Thus,

$$\begin{aligned} V_{\text{feed}}(x,s_0,q,t) &= \mathrm{e}^{-M}(1-\rho)F(x + \beta kx - \alpha x^{3/4} - \gamma s^{v} kx, s_0, q + kx, t+1) \\ &+ \mathrm{e}^{-M}\rho \sum_{j=1}^{3} \Pr(G_j | G_i, \text{WL}) F(x + \beta kx - \alpha x^{3/4} - \gamma s^{v} kx, \bar{s}_j, q + kx, t+1) \end{aligned} \tag{8}$$

If a caterpillar moves, the new glucosinolate concentration is conditioned on the concentration it moved from and movement distance. A caterpillar cannot feed at the same time that it moves. Therefore, the amount of damage on the plant, q, remains unchanged when a caterpillar moves within a plant. If a caterpillar moves between plants, the new plant is assumed to have no previous damage. However, because initial glucosinolate concentrations are determined from a distribution over all plant types, the new concentration may be equivalent to a previously damaged plant. The expected fitness for each scale of movement is therefore

$$V_{\text{moveWL}}(x,s_0,q,t) = \mathrm{e}^{-M}\sum_{j=1}^{3}\Pr(G_j|G_i,\ \text{WL})F(x-\alpha x^{3/4}-\mu_{\text{WL}}(x),\ \bar{s}_j,q,t+1) \tag{9}$$

$$V_{\text{moveBL}}(x,s_0,q,t) = \mathrm{e}^{-M}\sum_{j=1}^{3}\Pr(G_j|G_i,\ \text{BL})F(x-\alpha x^{3/4}-\mu_{\text{BL}}(x),\ \bar{s}_j,q,t+1) \tag{10}$$

$$V_{\text{moveBP}}(x,s_0,q,t) = \mathrm{e}^{-M}\sum_{j=1}^{3}\Pr(G_j|G_i,\ \text{BP})F(x-\alpha x^{3/4}-\mu_{\text{BP}}(x),\ \bar{s}_j,0,t+1) \tag{11}$$

For simplicity, I assumed the mortality rate, M, is constant for all behaviours.

RESULTS

Effects of variation on herbivore fitness and behaviour

The main result from these models is that variation in glucosinolate concentration reduced the predicted fitness of caterpillars, and adding more scales of variation reduced fitness further. Fitness was highest in the deterministic model (model A) and lowest in the model that included all scales of variation (model F). Different scales of variation affected different aspects of caterpillar fitness. Variation among plants (model B) and induction (model C) markedly reduced pupation rates by increasing the number of insects killed as a result of glucosinolates (Table 3). The ability of plants to induce caused a slight decrease in expected egg complement, but variation among plants had no effect on fecundity (Table 3). The other scales of variation had no effect on pupation rates. The average size at pupation was the same in all models (Table 3). This corresponds with experimental studies that have shown no difference in pupal mass due to plant defences, but have shown reduced survival or growth (e.g. Agrawal and Kurashige, 2003; A.L. Shelton, unpublished data).

Small-scale stochastic variation within leaf parts, included in model F, was the only scale of within-plant variation that significantly affected herbivore fitness. Model F predicted reduced fecundity, primarily as a result of increased larval durations leading to greater age at pupation (Table 3). Longer larval life spans have often been suggested to reduce herbivore fitness (Feeny, 1976; Clancy and Price, 1987; Fordyce and Shapiro, 2003). This result suggests that stochastic variation within leaf parts significantly reduces the long-term growth rate of caterpillars.

The behaviour of caterpillars in each model was also affected by the scales of variation included. Because model A is completely deterministic, the results were also deterministic.

Table 3. Values of traits correlated with fitness in each of the six models

Model	Smallest scale of variation	Percent pupation	Percent dead from defences	Percent dead from predation	Larval duration	Mean weight at pupation	Expected egg complement
A	none	86.1	0	13.9	15.9 (3.23)	247.8 (0.0)	263.6 (0.0)
B	plants	67.1	16.7	16.2	15.6 (10.67)	250.0 (0.2)	264.5 (20.5)
C	induction	53.9	33.3	12.9	15.2 (9.71)	249.6 (1.3)	250.4 (24.4)
D	leaves	56.2	27.8	16.0	15.4 (10.21)	249.7 (1.2)	249.8 (25.5)
E	leaf parts	54.8	30.4	14.8	14.6 (10.13)	249.8 (3.0)	250.3 (25.6)
F	within leaf parts	55.4	25.5	19.1	20.6 (12.44)	248.6 (7.8)	227.2 (32.5)

Note: All values are means (± standard deviation) from simulation of 1000 caterpillars. Each model includes all the scales of variation above it.

Caterpillars fed constantly and then pupated when their weight reached x_{max}. The uniform glucosinolate concentration (the average over all samples in the empirical data) was not high enough to be deleterious to caterpillars, and no caterpillars died from glucosinolates. In model B, individual plants differ but there is no induction or variation within plants. As a result, caterpillars fed on plants with low glucosinolate concentration and moved off other plants. Pupation rates were markedly lower than those in model A because some caterpillars encountered a series of plants with high defences. In model C, plants induce their defences in response to feeding by caterpillars. In addition to leaving plants with high initial glucosinolate concentrations, caterpillars also moved from plants after feeding for long enough to induce the plant's defences.

In models D–F, which include within-plant variation, caterpillars often moved within plants. In each of these models, caterpillars always fed on plants with very low initial glucosinolate concentrations ($s_0 < 3$) and never fed on plants with high concentrations ($s_0 > 7$). If induction level (q) was low, caterpillars moved between leaves in model D, where there is no variation within leaves, and within leaves in models E and F, which include variation within leaves. At intermediate initial glucosinolate concentrations, caterpillars moved from the plant when feeding had induced high concentrations, and fed otherwise (Fig. 1).

Sensitivity to parameters

I independently varied each of the parameters that I could not estimate directly from data to examine their effects on the results. The degree of small-scale stochastic variation, ρ, and detoxification costs had the strongest effects on predicted fitness and behaviour. Increasing values of ρ generally decreased both pupation rates and expected fecundity, but pupation rate increased slightly between $\rho = 0$ and $\rho = 0.2$ (Fig. 2).

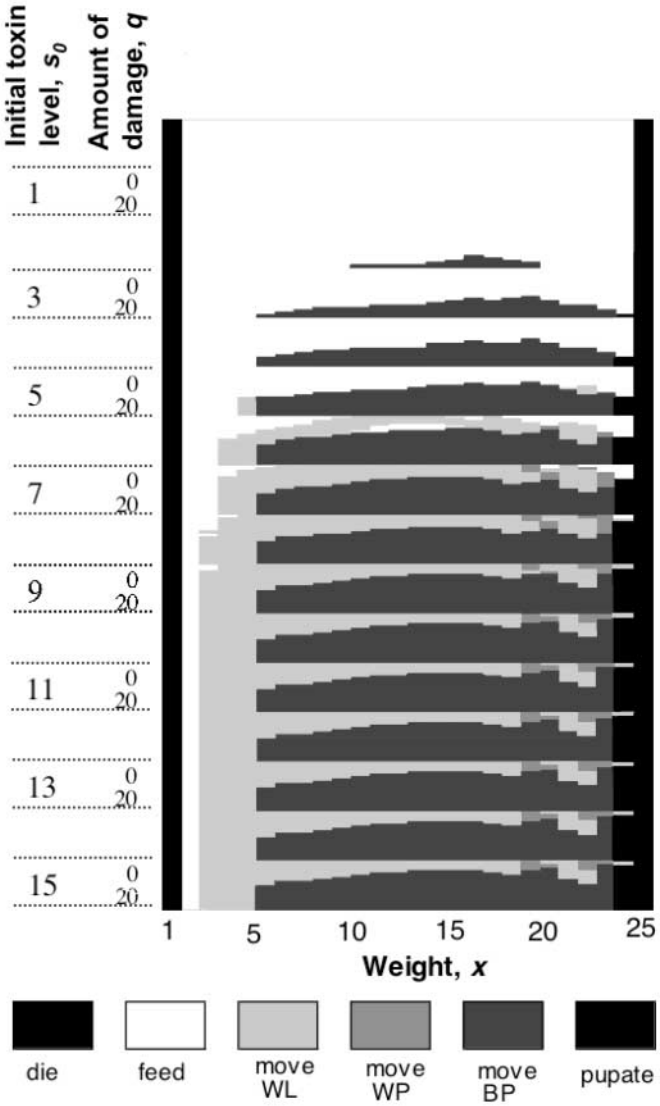

Fig. 1. Predictions from the backward solution of model F with the parameter values in Table 2. Each combination of weight (x), initial toxin level (s_0) and amount of feeding (q) is represented by a single rectangle. The glucosinolate concentrations of the plants are displayed along the vertical axis, sorted by initial concentration and then by amount of damage (i.e. degree of induction). Results shown are for time 1 but are identical to those for other times far from T_{max}. Black indicates death for $x < 10$ and pupation for $x \geq 10$.

D_{ratio} determines the proportion of total energy intake used to detoxify a low dose of glucosinolates. I used a relatively low value ($D_{ratio} = 0.05$) as a baseline. If I increased D_{ratio} to 0.1, small caterpillars died when they encountered a high glucosinolate concentration. At even higher detoxification costs, all caterpillars died on high glucosinolate concentrations.

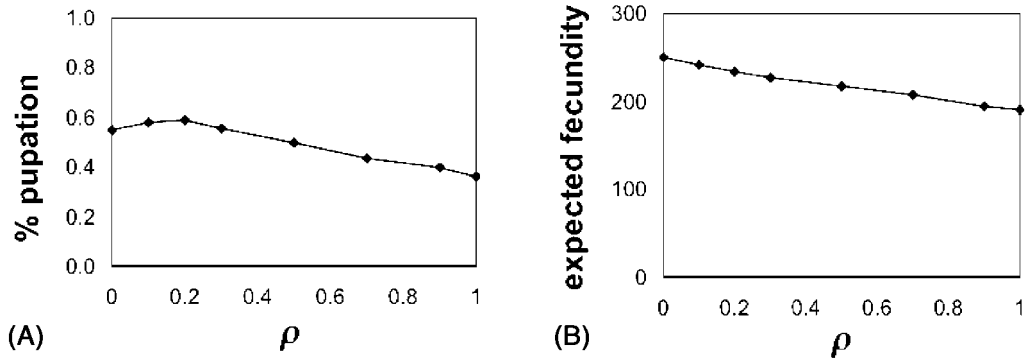

Fig. 2. Effect of within-part variability, ρ, on expected fitness of caterpillars. (A) Percent of 1000 simulated insects that pupated for different values of ρ. (B) Mean expected fecundity of caterpillars that pupated.

Trichoplusia ni caterpillars (and many other herbivores) are only mildly affected by glucosinolates (Agrawal, 2000), so low values may be more biologically reasonable.

The degree of non-linearity of detoxification costs, v, also had significant effects on model predictions, but the existence of non-linear detoxification costs is not essential to the model predictions. For the models reported above I used $v = 2$, where detoxification costs were greater for high glucosinolate concentrations but relatively low for low to moderate concentrations of glucosinolates, and 55% of caterpillars pupated. If $v = 1$, detoxification costs increase linearly with glucosinolate concentration. In this case, caterpillars always fed in place, regardless of glucosinolate concentration (with $D_{\text{ratio}} = 0.05$), and 84% survived to pupation. Linear costs of a higher magnitude ($D_{\text{ratio}} = 0.2$) produce predictions similar to those of non-linear costs of a lower magnitude. When $v = 3$, caterpillars only fed on plants with very low initial glucosinolate concentrations, and moved from plants with relatively low levels of damage. Only 18% of caterpillars survived to pupation, and 67% died of glucosinolate toxicity. In addition, as v increased, larval duration increased and expected fecundity decreased.

Other parameters had little effect on the predictions of the model. Larger metabolic costs slightly increased the sizes at which caterpillars moved, but when metabolic costs were very high, most caterpillars died or had extremely low fitness. Lowering movement costs caused caterpillars to move more often. Increasing movement costs had little effect. Changing the proportions of damaged plants, young leaves and lower parts of leaves in the environment had little effect on the predictions of the model except when all were increased to very high levels. Then, the caterpillars pupated at smaller sizes.

Comparing model predictions to data

To ensure that the model produces reasonable results and is well calibrated, I compared the predictions from model F, which includes all the chemical variation observed empirically, to experimental data of growth, movement and pupation rates of *Trichoplusia ni* caterpillars on *R. sativus*. Ensuring the models accurately predict these basic descriptions of behaviour and fitness strengthens the support for predictions about the importance of variation. To avoid circularity, I used a separate data set from that used to parameterize the model.

The predicted growth curves were very similar to those from the empirical data. Model caterpillars that pupated had approximately exponential growth, and those that died grew slowly and never got very large. Growth curves of caterpillars in experiments followed the same patterns and had similar maximum larval weights (data not shown). The time to pupation in the model was approximately half of that in experiments, because the model assumes caterpillars fed or moved constantly, never resting as real caterpillars do. In laboratory experiments, in which *Trichoplusia ni* caterpillars were fed pieces of leaves from *R. sativus* plants that varied in glucosinolate concentrations, 67% of caterpillars pupated. In the model, when I excluded death due to predation (as in the experiments), the pupation rate was 68.5%.

Movement rates were similar but not identical to those from empirical data. The model predicted less frequent within-plant movement than I observed in controlled field experiments. Model caterpillars moved an average (± standard deviation) of 1.47 ± 1.98 times within leaves, 0.01 ± 0.12 times between leaves and 0.95 ± 1.07 times between plants during their life spans. In an experiment in which I allowed third- to fifth-instar caterpillars to move freely among three *R. sativus* plants in a large container for 1 week, I observed an average of 2.25 ± 1.29 within-leaf moves, 2.0 ± 1.83 between-leaf moves and 0.94 ± 1.18 between-plant moves. In both the model and the experiment, most between-plant moves occurred when caterpillars were quite large.

DISCUSSION

The models described here indicate that variation in the concentration of chemical defences in plants, even when mean concentration is held constant, is probably deleterious to herbivores. The largest scales of variation examined (among plants and induction of glucosinolates within plants) and the smallest scale (variation within a part of a single leaf) had the greatest effects on predicted herbivore fitness. Large scales were detrimental because a caterpillar on a highly defended plant had to move to a different plant to escape the high toxin concentrations, and such moves are assumed to be costly. The importance of small-scale variation is surprising. Variation on this scale was deleterious because caterpillars experienced it as a stochastic change in the glucosinolate concentration while feeding. All other scales of variation were experienced only when a caterpillar made a choice to move. The greater unpredictability at this scale was based on empirical data on glucosinolate concentrations in the leaves of *R. sativus* (Shelton, 2002), which indicate a random pattern of small-scale spatial variation in glucosinolate concentrations within leaves.

These models may actually underestimate the importance of chemical variation. I assumed that caterpillars could detect the distribution of defences and behaved optimally in response. This may give caterpillars an advantage that they do not have in nature. Herbivores probably do not have perfect knowledge of the distribution of defences and may not always behave optimally. In addition, plants may adaptively adjust their defences in response to herbivores (Karban and Baldwin, 1997). Weakening these assumptions should increase the deleterious effects of variation on herbivores.

Large- and small-scale variation affected the fitness of caterpillars in different ways. Variation among plants and variation due to induction decreased pupation rates because more caterpillars died from glucosinolates. Induction also slightly decreased the expected fecundity of caterpillars. The pupation rates decreased because small caterpillars often lack

sufficient energy to make costly between-plant moves and may be stuck on low-quality plants. Many of these caterpillars died from continued exposure to high concentrations of glucosinolates.

In contrast, small-scale variation within leaves did not change pupation rates, but it reduced the expected fecundity of caterpillars. Because caterpillars cannot predict the small-scale spatial distribution of glucosinolates within leaves, the concentration in their food might spontaneously change from low to high. This uncertainty is generally deleterious to caterpillars. It results in lower overall growth and later pupation.

However, not all small-scale variation is bad. A small degree of variation within leaf parts appears to benefit caterpillars. Pupation rates were higher when there was a 10–20% chance that glucosinolate concentration would change within a leaf (ρ) than when within-leaf concentrations were constant (Fig. 2). When the probability of stochastic within-leaf variation was greater than 20%, expected pupation rates quickly declined. A small degree of spatial variation within leaves gives caterpillars a chance to escape a high concentration of glucosinolates without paying any cost for moving, but higher degrees reduce the caterpillars' chances of remaining on a high-quality patch of leaf.

The predictions about the effects of small-scale variation on herbivore behaviour are particularly interesting because few studies have addressed chemical variation at small scales within plants, and its effects on herbivore fitness and behaviour are largely unknown. Within-plant variation in quality may cause herbivores to move within a plant more frequently. This could result in increased metabolic costs from movement as well as increased risk of predation (Schultz, 1983). Increased herbivore movement might also result in a more dispersed pattern of damage. Mauricio *et al.* (1993) found that in *Raphanus sativus* dispersed damage had little effect, but the same amount of leaf damage in a more concentrated pattern significantly reduced total plant biomass and reproductive effort. Thus, within-plant variation may affect herbivore behaviour, and this altered behaviour might in turn affect plant fitness. This suggests that plant chemistry and herbivore behaviour can only be fully understood when examined simultaneously.

These models demonstrate that chemical variation may have important ecological effects, but variation may also have important evolutionary consequences. Variation within and among plants may be important for preventing the development of resistance in herbivore populations, especially insect herbivores that have short generation times compared with their plant hosts (Whitham and Slobodchikoff, 1981). Models by Gardner *et al.* (1998) and Gardner and Agrawal (2002) demonstrate that a variable dose of defence, either by subjecting herbivores to varying doses over time or through induction and relaxation of defences over time, can significantly increase the time required for development of resistance.

If chemical variation is an important aspect of a plant's defence, then selection should increase variation over time. For this to be possible, there must exist additive genetic variation for variation in chemical defence. Agrawal *et al.* (2002) measured genetic variation for induction in *Raphanus raphanistrum*. They found that plant families differed in their inducibility and there was significant additive genetic variation for induction. This indicates that it is possible for selection to lead to increasing chemical variation.

It is not known how variation at these different scales is maintained in plants or if it can be actively controlled. Recent work by Orians and colleagues demonstrates that the vascular architecture of plants can create variation of nutrients and plant defences within and among leaves of a plant. They found systemic induction in tomato plants was variable

among leaves due to differences in their vascular connections. When a single leaf was experimentally damaged, vertically aligned leaves, which have direct vascular connections, showed strong induction of proteinase inhibitors. Adjacent leaves with weaker vascular connections showed weaker induction and there was no induction in leaves opposite to the damaged leaf (Orians *et al.*, 2000). Another paper demonstrated that this vascular compartmentalization continues to the roots, resulting in nutrient sharing only among certain parts of a plant (Orians, 2002). This could cause small-scale patchiness in soil nutrients to create additional variation within a plant. Such vascular connections may explain, at least in part, the existence of chemical variation in leaves.

The results of these models indicate that variation in the distribution of chemical defences within and among plants can have important effects on herbivore fitness and behaviour even when the mean concentrations are constant. Plants may use variation in chemical defences to improve the effectiveness of their defence in two ways. First, they can increase concentrations in parts of higher value (e.g. young leaves) or higher risk (e.g previously damaged plants). Second, they can increase the stochastic variation of their defences to increase uncertainty about food quality for herbivores. My results indicate that both of these mechanisms, at different scales, may affect herbivores. In addition, similar patterns may occur at scales other than those I studied. Studies of plant–herbivore interactions may therefore need to pay greater attention to patterns of variation in plant defences and the effects of this variation on herbivores.

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APPENDIX: CALCULATION OF GLUCOSINOLATE CONCENTRATIONS

The probabilities of different glucosinolate concentrations were determined from the data in Shelton (2002). Glucosinolate concentrations varied according to previous damage, leaf age and leaf part. I used these three factors to define eight different leaf types. The frequency of each was determined from the frequency of damaged plants, young leaves and lower leaf parts (Table 2). Model F includes all the scales of chemical variation observed in the data; therefore, the probabilities of different glucosinolate concentrations in it were determined from the data directly. For all other models (A–E), the probabilities were determined by averaging over the scales of chemical variation that were excluded (see Table 1).

Calculations for model F

To simplify the probability model and to gain better accuracy in estimations of probabilities, I divided the observed glucosinolate concentrations into three categories: low, medium and high. I calculated probabilities for these three categories. I then selected a specific glucosinolate concentration from a uniform distribution within that category. The glucosinolate concentrations used in the model, s, were μmol glucosinolates per cm^2 of leaf area, divided by 4 to minimize the parameter space required (Table A1).

Table A1. Definitions of different scales used for glucosinolate concentrations in the model

Category	Actual glucosinolate value (μmol·cm^{-2})	Glucosinolate level in model	Glucosinolate category
Low	0–20	$0 \le s \le 5$	$G_i = 1$
Medium	20–40	$5 \le s \le 10$	$G_i = 2$
High	40–65	$10 \le s \le 15$	$G_i = 3$

Note: s represents the glucosinolate values used in the model and is equal to the actual values divided by 4. G_i is the category used in probability calculations.

The probabilities were calculated in two steps. First, because I assumed caterpillars did not know where they were on the plant, I calculated the probability the caterpillar was on each of the eight leaf types, given the glucosinolate category. I multiplied the result by the probability the caterpillar would move to a given glucosinolate category from that leaf type, given a particular distance of move. For example, the probability of moving to glucosinolate category G_j at $t+1$ from G_i at t for a within-leaf move is:

$$\Pr\{G_j|G_i,\mathrm{WL}\} = \sum_{m=1}^{8} \Pr\{l_m|G_i\}\Pr\{G_j|l_m,\mathrm{WL}\} \tag{A1}$$

where l_m is the leaf type the caterpillar is on at time t. The first term is solved by Bayes' Theorem (Ross, 1998) as

$$\Pr\{l_m|G_i\} = \frac{\Pr\{G_i|l_m\}\Pr\{l_m\}}{\sum_{m=1}^{8} \Pr\{G_i|l_m\}\Pr\{l_m\}} \tag{A2}$$

where $\Pr\{G_i|l_m\}$ is from the empirical data (Table A2) and $\Pr\{l_m\}$ is determined from the parameters $\Pr\{I\}$, $\Pr\{Y\}$ and $\Pr\{L\}$.

Table A2. Observed probabilities of glucosinolate concentrations determined from data on *Raphanus sativus*

Leaf type	n	Pr(Low)	Pr(Med)	Pr(High)
1. UOM	120	0.9250	0.0750	0.0000
2. UOL	36	0.8378	0.1622	0.0000
3. UYM	116	0.6897	0.3103	0.0000
4. UYL	38	0.6053	0.3684	0.0263
5. IOM	124	0.7177	0.2500	0.0323
6. IOL	32	0.5000	0.3750	0.1250
7. IYM	114	0.6372	0.2655	0.0973
8. IYL	39	0.3590	0.3333	0.3077

Note: Leaf types were defined by the combination of three treatments: toxins induced (I) or uninduced (U) by previous damage; young (Y) or old (O) leaf; main lobe (M) or lower leaflets (L) of leaf. Category boundaries are defined in Table 2. Data are from A.L. Shelton (unpublished).

The second term in equation (A1) is the product of the probability of leaf type l_n and the probability of moving to glucosinolate category G_j, given that the caterpillar moves to leaf type l_n at $t + 1$:

$$\Pr\{G_j|l_m,\mathrm{WL}\} = \sum_{n \in \mathrm{WL}_m} \Pr\{G_j|l_n\}\Pr\{l_n\} \tag{A3}$$

The set WL_m represents the leaf types to which the caterpillar can move given that it moves within a leaf from leaf type l_m. Because not all leaf types are accessible, the probability of each is divided by the sum of the probabilities of the potential leaf types:

$$\Pr\{f_l\} = \left(\frac{\Pr\{f_l\}}{\sum_{l \in \mathrm{WL}_k} \Pr\{f_l\}} \right) \tag{A4}$$

The probabilities for between-leaf and between-plant moves were calculated similarly, substituting the appropriate set of leaf types for each.

Calculations for other models

The same approach was used for model E, which excludes only stochastic variation within leaves. (This variation is included as the probability, ρ, that the glucosinolate concentration will change during feeding rather than as a result of movement.) Model A is completely deterministic, so the glucosinolate concentration for all parts of all plants is the grand mean over all leaf types and $s = 4.08$. The caterpillar gets this concentration regardless of where it moves or how long it feeds on a plant. Model B includes variation among plants but not variation within plants. Therefore, the glucosinolate concentration changes only if the caterpillar moves to a new plant. The probability of each glucosinolate category was determined by averaging over all leaf types (Table A3). Model C adds the effect of induction. So again a new glucosinolate concentration is encountered only in a move to a new plant, but the probability of each glucosinolate category was determined separately for damaged and undamaged plants (Table A3). Model D adds variation among young and old leaves. Probabilities for each glucosinolate category were calculated for each of four leaf types, averaging over leaf part (Table A3). Glucosinolate concentration can change when the caterpillar moves to a new leaf or a new plant.

Table A3. Probabilities of each glucosinolate category for null models

Model	Leaf types	n	Pr(Low)	Pr(Med)	Pr(High)
B	All	619	0.7044	0.2439	0.0517
C	U	311	0.7878	0.2090	0.0032
C	I	308	0.6201	0.2792	0.1006
D	UO	157	0.9045	0.0955	0.0000
D	UY	154	0.6688	0.3247	0.0065
D	IO	156	0.6731	0.2756	0.0513
D	IY	152	0.5658	0.2829	0.1513

Note: Values were calculated from the data in Table 3 by summing over different leaf parts. I = induced, U = uninduced, O = old leaf, Y = young leaf. Toxin categories are defined in Table 2. In model A, all plants had a toxin level equal to the average for all samples combined, $\bar{g} = 16.3205\ \mu\mathrm{mol}\cdot\mathrm{cm}^{-2}$ or 4.0801 in units of s.