On the evolution of plant secondary chemical diversity

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SUMMARY

A common-sense evolutionary scenario predicts that well-defended plants should have a moderate diversity of secondary compounds with high biological activity. We contend that plants actually contain a very high diversity of mostly inactive secondary compounds. These patterns result because compounds arising via mutation have an inherently low probability of possessing any biological activity. Only those plants that make a lot of compounds will be well defended because only high diversity confers a reasonable probability of producing active compounds. Inactive compounds are retained, not eliminated, because they increase the probability of producing new active compounds. Plants should therefore have predictable metabolic traits maximizing secondary chemical diversity while minimizing cost. Our hypothesis has important implications to the study of the evolution of plant defence.

1. EVOLUTION OF WELL-DEFINED PLANTS: A COMMON-SENSE SCENARIO

Commonly accepted facts and notions about plant secondary metabolites can be used to construct a common-sense scenario for the evolution of well-defended plants. Compounds arising via enzyme mutations adversely affect herbivores and plant pathogens (Rosenthal & Janzen 1979; Horsfall & Cowling 1980). A diversity of active compounds confers greatest resistance because mixtures of compounds enhance activity and because plants interact with many organisms (Jones 1983; Berenbaum 1985). Because production of compounds incurs energy, material and opportunity costs (McKey 1979; Rhoades 1979), diversification is constrained once a plant is well defended. At the same time, consumer adaptation (i.e. avoidance, detoxification and utilization of compounds; Chapman & Blaney 1979; Brattsten 1979; Bernays & Woodhead 1982) and new colonists will tend to decrease activity of compounds and overall defensive efficacy, with highly active compounds selecting for rapid consumer adaptation (Pimentel & Belotti 1976). Plants that eliminate inactive compounds that are not essential precursors of active compounds will incur reduced costs without any decreased benefits. Further mutations produce new, active compounds, replacing those that have been eliminated. If natural selection favours plants that are well defended but minimize cost, well-defended plants should contain a moderate diversity of highly active compounds, and few if any inactive compounds other than essential precursors. Is this what we find?

2. OBSERVED PATTERNS OF ACTIVITY AND DIVERSITY

(a) *Are most secondary compounds highly active?*

Some highly active compounds are known to occur in plants (e.g. azadirachtin; pyrethroids, nicotine; Jacobson & Crosby 1971). However, most active compounds are rarely 100% lethal or inhibitory at naturally occurring concentrations. Although it is likely that most plants contain a few active compounds, the number of active compounds is a small percentage of all the compounds that have been isolated. Programmes screening plant extracts or pure natural products have a very low frequency of discovery of highly active compounds, irrespective of whether the screening was against consumers or other targets (i.e. cancer–anticancer, medicinal, plant growth regulator). It is a 'basic dogma of random screening that only a very small percent of samples should be active' (Suffness & Douros 1979, p. 81; Goodman & Gilman 1965). For example, in the National Cancer Institute anticancer screening programme, only 4.3% of plant species had any activity, and pure compounds with high activity resulted from 0.07% of species (Suffness & Douros 1982). Rohm and Hass estimate that 1–2% of plants have activity (C. Swithenbank, personal communication), whereas Rothhamsted programmes find about 3% of plants to be active against insects (J. A. Pickett, personal communication). Of 7000 isolates of *Bacillus thuringiensis* from soil that were screened against grass grub, only 7% had any detectable activity and only 0.07% had what could be termed high activity (P. Wigley & C. Chilcott, personal communication).

The synthetic organic chemist is no better at finding activity by screening. Commercial programs are constrained by requirements other than activity *per se*; nevertheless, Dupont estimates that about 0.005% of compounds screened for insecticidal, developmental or antifeedant activities result in a commercial product (J. Frazier, personal communication). Ciba-Geigy observe that random or directed screening with synthetic and natural products have about the same very low

success rate (H. Fischer, personal communication; Geissbühler *et al.* 1982).

(*b*) *Why are so many compounds inactive and why are they retained?*

Is the prevalence of inactivity solely a consequence of rapid consumer adaptation? We think not. Screening programmes with synthetic or natural compounds never encountered by particular consumers, and screening of natural products for biological activities not relevant to consumers (e.g. plant-growth regulators) reveal the same pattern of a low frequency of occurrence of biological activity.

If most compounds are relatively inactive, why are they not eliminated? The common-sense scenario predicts elimination of inactive, inessential (non-precursor) compounds because they incur cost with no benefit. Does retention of inactive compounds mean that the cost of defence is non-existent or unimportant? This seems unlikely, given the many cogent arguments and circumstantial lines of evidence to support a notion of cost, even if we do not know how great the cost is (e.g. trade-offs between inherent growth rate and resource availability, defence and growth (Bryant *et al.* 1983; Coley *et al.* 1985); inducible defences (Tallamy & Raupp 1991)).

(*c*) *Why are there so many secondary compounds?*

Most plants contain a very high diversity of secondary metabolites. For example, both carrot and bracken fern have 12 biosynthetically distinct pathways, and bracken has at least 27 different structures within the class sesquiterpene indan-1-ones (Jones 1983; Jones & Lawton 1991). It is reasonable to assume that the observed diversity is necessary to be well defended (Haldane 1949). Nevertheless, it appears that plants contain many more compounds than indicated by the common-sense scenario. If true, why do plants produce so many compounds rather than a few? How has this diversity arisen? How is it maintained? How does this diversity relate to the observation that most compounds are inactive?

3. THE INHERENT PROBABILITY OF BIOLOGICAL ACTIVITY

We believe that the discrepancies between expected and observed activity and diversity in the common-sense scenario are due to a hidden assumption that is incorrect. Compounds arising via mutation are assumed to have an inherently high probability of possessing biological activity against organisms with which the plant interacts. Instead, we postulate that the inherent probability is low. This is a fundamental toxicological property of the interaction between chemicals and organisms. The low success rate reported from screening programmes shows that toxicologists are acutely aware of a phenomenon that plants have had to deal with for millions of years. Although it is interesting to speculate why low activity is a fundamental toxicological property, it is not necessary to know the reasons in order to examine the consequences to plant defence.

4. AN EVOLUTIONARY SCENARIO BASED ON A LOW PROBABILITY OF ACTIVITY

If secondary metabolites have an inherently low probability of possessing biological activity, mutations that add active compounds and increase defence will be rare. Plants producing only inactive compounds incur costs with no defensive benefit and should be selected against. Some plants will have a few active compounds and be well defended. Consumer adaptation and new colonists will then decrease defensive efficacy. As the probability of producing new active compounds is low, how can plants maintain adequate defence in the face of consumer adaptation and colonization?

Consider what would happen if a well-defended plant with a few active compounds retains some inactive compounds. This could occur if the rate of enzyme mutations adding compounds temporarily exceeds the rate of deletion of compounds, and if the cost of retaining these compounds is not excessive (i.e. cost–benefit remains within selectively advantageous limits). The total number of compounds added or deleted over time will be a function of the mutation rate per biosynthetic step (compound) multiplied by the total number of biosynthetic steps (compounds). Thus plants with a high diversity of compounds, irrespective of their activity, have a greater probability of adding or deleting compounds over time than plants with a low diversity of compounds. Because the probability of any compound having activity at any time is low in both cases, it is clear that plants with a high absolute diversity have a greater probability of producing one or more active compounds at any time than plants with a low diversity.

Plants with a high diversity of compounds will 'screen' a greater number of compounds at any time and a greater variety of compounds over time than plants with a low diversity. We argue that this is the only effective 'strategy' if the 'screening success' is inherently low. It is also a 'strategy' that produces the observed patterns of well-defended plants containing a high diversity of mostly inactive compounds and a few active compounds.

Maintenance of high diversity therefore increases the probability of production of new active compounds in the face of consumer adaptation. A high diversity can also increase the probability that selection for consumer adaptation to one active compound may then lead to susceptibility to another, previously inactive compound (see, for example, Gould 1988). Furthermore, because plants are continually subject to attempted colonizations by new consumers (Jones 1983; Strong *et al.* 1984), plants with a high diversity of compounds also have a higher probability of possessing a compound active against potential colonists.

5. METABOLIC TRAITS MAXIMIZING DIVERSITY AND MINIMIZING COST

Maintenance of a high diversity of secondary chemicals is therefore advantageous, but it probably incurs costs. We would therefore expect natural selection to favour plants that possess metabolic traits that maximize diversity and minimize cost. What would such traits look like and do plants possess them?

(*a*) *Branched pathways*

Branched pathways of secondary metabolism should be relatively common and simple linear pathways should be rare. Continual bifurcation (i.e. mutation at a biosynthetic step results in production of a new product and retention of the existing product) results in exponential doubling in the number of compounds over time at a constant mutation rate per biosynthetic step, compared with additive increases in diversity with linear pathways (i.e. mutation at a biosynthetic step results in production of a new product and deletion of the existing product). For example, after only four mutations a bifurcating pathway will have 1.8 times as many compounds as a linear pathway. Deletion mutations are likely to have less of an effect on diversity in complex versus simple pathways. For example, in a linear pathway with six biosynthetic steps (i.e. seven compounds), a single random deletion mutation has an equal probability of removing either one, two, three, four, five or six compounds. However, in a bifurcating pathway with the same number of steps and compounds, a single mutation has four chances of removing only one compound and two chances of removing three compounds. Similarly, an active compound may have a lower probability of being eliminated by a single mutation in a bifurcating than in a linear pathway. Thus in the above example, an active compound at the end of the linear pathway will be removed by a deletion at any of the six steps; whereas only two of six possible deletions will eliminate the active compound if it is at one of the four terminal points of a bifurcating pathway.

Highly branched pathways are one of the most striking characteristics of secondary metabolism (Mann 1987). For example, pathways for biosynthesis of sesquiterpenes, alkaloids, anthraquinones and shikimate derivatives are all highly branched (Bu'lock 1965; Iwasaki & Nozoe 1974).

There are two further consequences of branched pathways. First, as the diversity of a pathway increases, a greater proportion of the total diversity is found in any one pathway. Thus, diversity of compounds within a class is always likely to exceed that between classes, as is observed (e.g. bracken; Jones 1983). Second, a new mutation is far more likely to add compounds to an existing pathway than start a new pathway from a primary metabolite, simply because the number of biosynthetic steps located in existing pathways is much greater. Consequently, there will be a high degree of canalization in pathway diversification, leading to the high degree of gross chemotaxonomic affinity that we observe in plants, despite the fact that there are clear differences in specific compound structure between related plants (see Harborne 1977).

(*b*) *Matrices and grids*

Metabolic matrices and grids should be common. Here a few enzymes convert a diversity of structurally similar precursors to many end products. Such matrices and grids confer the same advantages as bifurcating pathways, but can also result in substantial cost savings. For example, in a four-step matrix with four precursors, 20 compounds are produced with only four enzymes. Matrices and grids are common in secondary metabolism. For example, biosynthesis of polyketides, carotenoids, xanthophylls, flavanoids, coumestrols, and alkaloids; cinnamic acid conversions and formation of coumarins and cinnamoyl quinic acids all involve grids and matrices (Bu'lock 1965; Hahlbrock & Grisebach 1975).

(*c*) *Combining pathways*

Combination of different pathways to produce new products is another means of increasing diversity while minimizing cost. Given an existing cost commitment to produce two pathways, biosynthesis involving products from each pathway will further enhance diversity at relatively little extra cost. Compounds of mixed biosynthetic origin are common. For example, mycophenolic acids, cannabinoids, isoprenoid quinones, furanocoumarins, furanoquinolines, alizarin anthraquinones, and certain flavanoids and alkaloids are all formed this way (Mann 1987).

(*d*) *Pathway regulation*

Most compounds in secondary metabolic pathways are produced in trace amounts, with one or a few components being quantitatively dominant (e.g. bracken sesquiterpenes, Jones & Firn 1979; monoterpenes, Gershenzon 1991). Major and minor components can occur in concentrations that differ by three or four orders of magnitude. Minor components have the potential to be just as active as a major components because the doses at which different compounds affect the same organism, and the dose at which the same compound affects different organisms can both vary by orders of magnitude. Thus a plant may obtain the same degree of defence from less costly minor components as more costly major components. Furthermore, mutations in the regulation of pathways, as a result of alterations in promoter or regulator genes and enzyme–substrate affinities, can potentially result in a new quantitative profile. Major components could become minor components and vice versa. This could result in marked shifts in biological activity with no real change in cost. The fact that closely related species or genotypes can possess the same compounds but have substantially different quantitative profiles (e.g. monoterpenes in conifers; Cates & Redak 1988) indicates that such shifts are relatively common.

Similarly, pathway regulation may be altered so that virtually all of the compounds are expressed in trace amounts. This maintains diversity and the

potential for activity, while reducing the material and energy costs. The phytoecdysteroids of bracken fern that we previously termed 'redundant defences' (Jones & Firn 1978) may conform to this pattern. A logical extension of this idea is inducible pathways. Here compounds are only produced following a particular signal associated with consumer attack, and operate over relatively short time frames. Such inducible pathways may substantially reduce material and energy costs without jeopardizing defensive capacity. Inducible pathways are widespread in plants (Tallamy & Raupp 1991).

(*e*) *Hidden pathways*

It is possible that mutations to regulator or promoter genes, or any type of mutation that deletes the first step in a pathway, could result in an entire pathway no longer being expressed, with substantial savings in energy and material costs. However, the genetic or biosynthetic machinery still remains, with the capacity to re-express the entire pathway after subsequent mutations. Plants may therefore contain hidden pathways. Carrot, a well-studied plant, produces minute traces of methyl sulphides, compounds characteristic of the totally unrelated onion family, and not reported for any other umbellifer (Jones & Lawton 1991). This suggests that carrot has the capacity to produce onion compounds, given a suitable regulatory mutation. We suspect that this type of phenomenon will turn out to be widespread in plants. If true, it would indicate that phenotypic characteristics of secondary metabolism may grossly underestimate the genotypic potential for chemical diversity. The observed chemotaxonomic affinities of plants may be just as much a consequence of failure to express pathways common to many unrelated taxa, as it is a consequence of possessing pathways only found in related taxa.

(*f*) *Reaction mechanisms*

Several other metabolic traits related to conversion reactions could promote diversification or decrease cost. First, we would expect that many of the enzymes of secondary metabolism would show relatively low substrate specificity. Such 'sloppy enzymes' could convert a range of structurally similar substrates into slightly different products; or do the same functional transformations on structurally dissimilar substrates, such as hydroxylation or methoxylation. Here the cost of many different transformations is reduced because only one enzyme is involved. Second, we would expect many isozymes. Mutations in genes coding for a particular enzyme could result in an isozyme with reduced specificity that then converts substrates that were not previously utilized, or produces different products from the same substrate. Isozymes of enzymes that do more than one transformation might result in products where only one of these transformations had occurred. We would expect 'sloppy enzymes' and isozymes to be important in branched pathways (see §5*a*). There is some evidence for sloppy substrate specificity and isozymes, particularly in metabolic matrices and grids (Bu'lock 1965); biosynthesis of phenolics (Neish 1964; Hahlbrock & Grisebach 1975) and reticuline and other alkaloids; oxidation of carotenoids, polyketide transformations, flavanoid hydroxylation and polymeric isoprenoid formation (Bu'lock 1965).

Third, we would expect polymeric assembly of secondary metabolites. Here, multiple classes of compounds are produced by sequentially combining different numbers of base units, with substantial savings in enzyme costs. Terpene biosynthesis is the classic example of polymeric assembly (Bu'lock 1965).

Lastly, we would expect that non-enzymic, chemical reactions would be important. Here many variants of a structure can be produced without any enzyme costs. Changes in cellular or vacuolar pH and ionic strength, for example, may facilitate such conversions as radical coupling and shiff base formation (Luckner 1972). Monoterpene and phenolic biosynthesis are good examples (Bu'lock 1965; Templeton 1969; Herbert 1981).

6. TESTING THE THEORY

We have presented some evidence in support of our ideas about the relations between plant defence, biological activity, chemical diversity and cost of defence. However, these ideas clearly require further evaluation and testing.

(*a*) *General patterns of diversity and activity*

The general patterns that are predicted by our theory are: (i) Most secondary plant compounds will have relatively low activity or no activity against a given organism; highly active compounds will be rare. (ii) Active compounds will have a relatively narrow spectrum of specificity because high activity requires receptor site specificity. (iii) Thus, highly active, broad-spectrum compounds will be very rare. (iv) Most plants will contain a few compounds active against organisms with which the plant interacts and organisms with which the plant has never interacted. (v) Well-defended plants will have both a higher absolute chemical diversity and a greater proportion of active to inactive compounds than poorly defended plants.

There are several problems with testing these general predictions. For example, it is hard to define what is meant by a well- versus a poorly defended plant. If plant defence is a zero-sum game, i.e. only more or less well-defended plants survive, there may be no poorly defended plants available for comparison. Furthermore, it is hard to estimate how many active compounds are necessary for good defence, or how many compounds are needed before an active compound is produced; i.e. there is no clear null model. Nevertheless, there are three approaches that could facilitate testing these general predictions. First, a more thorough analysis of existing data on activity and diversity is needed, to more clearly define patterns.

Second, only a small fraction of all plants and a small fraction of the compounds they contain have been tested against a small fraction of the consumers with which a plant interacts or could interact. Thus, a

few comprehensive studies are needed that more fully characterize the chemical diversity of selected plants. Most of the compounds they contain should be screened at naturally occurring concentrations, singly and in admixture, against most of the organisms with which a given plant interacts (i.e. both adapted consumers and potential colonists; for example, see Jones & Firn (1979)).

Third, mathematical or simulation approaches may be of value in generating null models. Such techniques could examine diversity–activity relations as a function of variable values for inherent probability of activity, mutation rates adding and deleting compounds, rates of consumer adaptation and costs of defence.

(*b*) *Metabolic traits*

Similar approaches could be taken for examining relations between diversity and activity at the metabolic level. For example, comparison of known pathway structures between primary and secondary metabolism, and between highly diversified and less diversified classes of secondary metabolites might reveal clear differences in the degree of bifurcation or matrix and grid formation, and frequency of pathway recombination. Modelling may facilitate comparisons between actual pathway structures and predicted structure based on maximizing diversity, minimizing the probability of loss of active compounds, and minimizing the cost of production.

There is a major advantage to examining predicted metabolic traits, as opposed to general patterns. Modern techniques of molecular biology could be used to investigate the regulation and expression of secondary metabolism. For example, what is responsible for quantitative differences in product expression of the same compounds in closely related plants? Why are some pathways expressed in trace amounts? What is the genetic basis of controls of inducible versus constitutive pathways? Does alteration of promotor or regulator genes result in expression of hidden pathways? Can such genes or genes for single enzymes be inserted into unrelated plants, causing expression of entirely new pathways or compounds? How substrate-specific are the enzymes of secondary metabolism? How many of these enzymes exist as multiple isozymes with different catalytic properties? Why are some pathways formed by polymeric assembly whereas others are not? How widespread are non-enzymic (i.e. chemical) conversions, and what determines whether chemical conversions can occur?

7. IMPLICATIONS

(*a*) *The cost of plant defence*

Cost is inherently difficult to estimate because secondary metabolism is an integrated component of whole-plant function. Compounds may protect against abiotic stress or damage and may serve both primary metabolic and defensive roles (Chew & Rodman 1979). However, our theory suggests that it may not be possible to obtain realistic estimates of cost, irrespective of these confounding factors. A number of attempts have been made to estimate cost based on a single compound or class of compounds that are active against one or a few consumers (see, for example, Krischik & Denno (1983); Coley (1986); Baldwin *et al.* (1990)). Because this approach assumes that these compounds constitute the defence of the plant, it is likely to grossly over- or underestimate the real aggregate cost of defence, depending on whether these compounds are major or minor components of the entire chemical diversity with all its attendant metabolic pathways and controls. It is quite likely that a different estimate would be obtained if different compounds active against different consumers were chosen.

Calculations that do not make assumptions about which compounds are active, but are based on the quantities of components, are more likely to produce accurate estimates of cost. However, such estimates (see, for example, Gulmon & Mooney (1986)) are usually based on the major components and their attendant pathways, and will tend to underestimate cost by excluding the cost of the host of other components comprising chemical diversity. This would be particularly true if most plants had a much greater diversity than is currently reported. Studies on the chemical diversity of the British umbellifers (Jones & Lawton 1991) reveal that the diversity of biosynthetically distinct pathways of secondary metabolites, let alone chemical diversity *per se*, is strongly positively correlated with the intensity with which the plants have been studied. Because most plants are not well studied, most estimates of cost based on component concentrations will be underestimates.

Cost estimates are of interest because cost–benefit relations are of paramount importance in evolutionary theories (McKey 1979; Rhoades 1979). A central part of our theory is that mechanisms must have evolved that ensure generation and retention of chemical diversity. Retention of inactive compounds is one way of increasing chemical diversity and the probability of producing active compounds in the face of consumer adaptation. Yet traditional views of cost–benefit demand that such compounds be lost by selection. This problem may be resolved if selection has favoured plants possessing general metabolic traits for generating diversity, and if the cost of retaining low concentrations of large numbers of inactive compounds is sufficiently low to be selectively neutral, or such costs are exceeded by benefits incurred by the increased probability of producing active compounds. Interestingly, the immune system of animals is an analogous means of generating massive chemical diversity at minimal cost. However, our understanding of the genetic and biochemical mechanisms of plant defence lags well behind our knowledge of the animal immune system.

(*b*) *The evolution of plant defence*

Our ideas have a number of important ramifications to theories on the evolution of plant defence. First, the presence of a high diversity of mostly inactive compounds implies that the relation between the specific chemical composition of a plant and its overall degree of defence will be weak, at best. Hence, the relations

between contemporary chemistry and past biotic interactions may be even weaker. Many, perhaps most of the chemicals may not be serving a specific role, and may never have served any role, other than their general contribution to the diversity necessary to increase the probability of having a few active compounds. Given sufficient diversity, the probability of isolating an active compound is actually quite high. It may therefore be erroneous to assume that this compound, or the class to which it belongs, specifically evolved as a consequence of selection by any particular organism(s). A compound found to be active against an organism in one plant species, and found to be widely distributed in related plants, does not necessarily imply that such compounds specifically evolved as defences and were followed by subsequent speciation. It may be more parsimonious to assume that as diversification will most likely proceed within a class (see §5*a*), there is a high probability of chemical similarity that may operate independent of biological activity.

Second, correlations between the quantity or quality of secondary metabolites and consumer abundance or damage are highly likely to result in spurious statistical relations, simply by chance. This is because at high diversity there are many degrees of freedom in the independent chemical variables.

Third, it may be erroneous to assume that compounds in plants are inactive solely because of consumer adaptation. Furthermore, even if we were able to show a specific metabolic adaptation in a consumer that was lacking in other related organisms, it cannot be assumed that the inactive compound serves no purpose. Such a compound may be inactive now but active in the past; may be active against some other organism; and, most importantly, may have been inactive in the past, but via mutation, produced an active compound.

Fourth, conclusions drawn from artificial selection experiments that show that concentrations of an active chemical can be increased, or that composition of a class of chemicals can be shifted towards greater activity may be misleading if they assume that natural selection will produce the same result. Without detailed knowledge of the controls over diversification, it may not be possible to guess the likely path of chemical evolution.

Last, and most important, the general metabolic traits that confer diversity may have been selected for very early in the evolution of plants, perhaps before the evolution of terrestrial plants. Microorganisms and algae show very similar traits, which either implies convergence or perhaps an ancestral characteristic that was selected for in the earliest organisms. However, once these traits were present, the 'natural screening process' of diversification and elimination may proceed in a manner that was more or less independent of specific biotic interactions, provided that there was always selection for well-defended plants. This does not mean that canalization of metabolism has not been influenced by specific biotic interactions, but it does imply that such canalizations may not be critical to the evolution of most well-defended plants.

Thus, our theory can be considered a null model for the evolution of plant defence. Tight or diffuse coevolution (Ehrlich & Raven 1964; Fox 1981; Berenbaum 1983; Futuyma 1983) or even sequential evolution (Jermy 1984) may not be necessary. We envision a world in which the probability of biological activity is low; in which some damage to plants is always inevitable; in which consumer organisms can always adapt, often rapidly; and in which the composition of the consumer community is constantly changing. At its simplest, the sole requirement for plant defence under these conditions may be the continual production of a diversity of secondary metabolites. The evolution of plant defence may therefore have proceeded independent of consumer adaptation, once these fundamental traits were in place.

We thank John Lawton, Centre for Population Biology, for helpful discussion; the British Ecological Society for a Travelling Fellowship (C.G.J.), the Royal Society, CIBA Foundation (C.G.J. and R.D.F.), and the Mary Flagler Cary Charitable Trust for support (C.G.J.). Contribution to the program of the Institute of Ecosystem Studies.

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Discussion

S. B. Malcolm (*Department of Biology, Imperial College, Silwood Park, Ascot, U.K.*). I think one can argue the converse of Dr Jones's suggestion that plants maintain a high diversity of potential chemical defences against herbivores because of the low probability that new chemicals will be biologically active. Instead it may be possible that low probability will select for plants to exploit single metabolic pathways that have a high probability of producing biologically active chemicals. That taxonomically similar groups of plants rely to a large extent on single kinds of chemical defences argues for such 'molecular parsimony' and not evolutionary 'expensive' diversity to counteract low probabilities. Diversity of chemical defences can be covered by a single molecular type. For example, the toxic cardenolides of foxgloves, oleander and milkweeds, in three different families, are steroids with the same kind of toxic activity. Variation of this single molecular pattern appears to account for most of the defensive needs of the plants. These include the full range of toxic/digestibility reducing and mobile/immobile defences suggested by apparency and resource availability hypotheses. The reason that this single molecular pattern can cover the full range of defensive needs is that cardenolide toxicity is targeted at disrupting a fundamental physiological attribute of all herbivores: Na/K ATPases. The use of coumarins by umbellifers, alkaloids by Solanaceae, glucosinolates by Cruciferae, and cardenolides by Asclepiadaceae argues for molecular parsimony and selection to increase the probability that new molecules will be biologically active.

C. G. Jones. Is there clear evidence that 'taxonomically similar groups of plants' do 'rely to a large extent on single kinds of chemical defence', as a means of solving the problem of an inherently low probability of activity? First, the diversity of secondary metabolic pathways is not known for most plants irrespective of whether or not such compounds are biologically active. Well-studied species or genera (e.g. bracken, British umbellifers) have a demonstrably high pathway diversity. Perhaps the perceived lack of pathway

diversity is because phytochemists tend to focus on particular classes of compounds in order to identify new structural variants; and chemotaxonomists often use these same variants to discriminate plants within related taxa. Second, perceived 'molecular parsimony' of defence may be an artifact of the tendency for studies on plant resistance to focus on activity of one or a few compounds against one or two organisms – usually dominant herbivores or major pests – rather than the much larger number of chemicals, herbivores and pathogens that may be involved in these interactions. More intensive studies (e.g. on bracken) reveal that resistance to a community involves different compounds within and between pathways. Thus it can be argued that the notion that the umbellifers use only coumarins, the Solanaceae use only alkaloids; the Cruciferae use only glucosinolates; and the Asclepidaceae use only cardenolides as defences is a reflection of study biases and inadequate data, rather than real 'molecular parsimony'.

From our toxicological perspective it is not possible that a 'diversity of chemical defenses can be covered by a single molecular type'. Screening studies show substantial differences in biological activity of the same or different compounds within and between target organisms. This is owing to both variation in the mode of action (i.e. which binding site or compound–compound interaction is involved) and variation in affinity (i.e. degree of binding to a particular site. This is equivalent to the dose required to effectively block all the receptor sites of a given type). Structurally dissimilar compounds usually bind to different types of receptors. Structurally similar compounds with the same mode of action can vary by orders of magnitude in their affinity to the same receptor site. Variation in receptor site structure between target organisms results in orders of magnitude variation in the degree of binding of the same compound. Consequently, from our toxicological perspective, a 'single molecular type' does not produce a diversity of different types of biological activities. The example of the common mechanism of cardenolides against certain Lepidoptera via Na/K ATPase inhibition supports our argument. Here, structurally similar compounds are not diverse defences, because only a single mode of action is involved. Nevertheless, variation in affinity does result in a different dose being required before biological activity is observed.

From our toxicological perspective, we do not distinguish between compounds termed 'toxic' versus those termed 'digestibility reducing' properties as in apparency theory. This distinction arose from the observed net effects on herbivores; reduced survivorship = 'toxic' and reduced growth = 'digestibility reducing'. We consider these terms to be toxicologically misleading because: (i) they equate net effects on herbivores to mode of action, when binding sites have not been determined; (ii) they emphasize differences between these two types of net effects, when toxicological studies show that both reduced survivorship and reduced growth can be end results of many different modes of action; (iii) they classify compounds such as tannins into one functional class (of 'digestibility reducers') that may not be justifiable toxicologically and (iv) they confuse mode of action with affinity (i.e. low dose required = 'toxin'; high dose required = 'digestibility reducer'). Whereas low dose required means high affinity and high dose required means low affinity, for the same mode of action. Toxicologically speaking, compounds vary in both mode of action and affinity (dose response) and the combination of these two characteristics results in net effects on herbivores. The relations among modes of action, affinity and net effects such as survivorship or growth have yet to be established. The terms 'mobile/immobile' refer to biosynthetic turnover of compounds and not biological activity. Tannins may be 'immobile' and monoterpenes may (or may not) turnover (i.e. be 'mobile'). However, the relation between these biosynthetic characteristics and biological activity has not been clearly established.