COMMUNITY STRUCTURE OF SECONDARY COMPOUNDS IN PLANTS

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ABSTRACT

It has been suggested that secondary substances in plants form effective defences against herbivores. This hypothesis can be tested through experimental field and laboratory studies. For example, it has been shown that non-protein amino acids in the seeds of certain legumes reduce their susceptibility to attack by bruchid seed-eating beetles. These and similar observations are discussed in relation to the development by individual species of biochemical strategies needed to ensure survival in a community.

INTRODUCTION

The primary role of secondary compounds in higher plant vegetation and seeds is defence against herbivores and micro-organisms. The data and circumstantial evidence in support of this hypothesis[1-10] are adequate to justify it as a working hypothesis. It is not fruitful to debate, at this time, the overall phenomenon of whether plants are protected or animals are affected by such secondary compounds. The discussion of secondary compounds that serve as animal attractants in fruits and flowers is intentionally avoided.

Acceptance of the defensive role of secondary compounds allows us to turn our attention to the ecological significance of the highly patterned heterogeneity in space and time displayed by secondary compounds. It also stresses the concept that secondary compounds themselves are no more useful in supra-specific chemotaxonomy than are such traits in animals as fur colour, tooth length, pupil diameter, etc. Being an ecologist, I cannot resist noting that adopting such an attitude defines our efforts as the study of community structure of secondary compounds. In this community, the individuals are molecules, the species is a population of molecules of a single kind, and the community is the plant and its neighbours, be they competitors, herbivores, mutualists or neutral. It is easy to recognize, then, that the processes that generate patterns in secondary compound community structure are the same as those that generate patterns in communities of whole organisms: natural selection, size of the resource base, competition, synergism, historical accident, and so on.

The structure of secondary compound communities is displayed in two quite different ways. On the one hand, animals certainly do not wander through the habitat feeding on all plants; the ecologist usually sees herbivores

eating or avoiding plants as though there are well defined and predictable patterns in the occurrence of secondary compounds. On the other hand, we have a large and ever-growing body of reports from the pharmacologist, biochemist, plant taxonomist, and natural-products chemist that this or that secondary compound occurs in this or that plant or plant part. The task lies in uniting these two bodies of information; this will undoubtedly generate hypotheses of sufficient generality that their testing will require the skills of the chemist and the ecologist working together[1, 5, 7–11].

As an ecologist, I wish to describe briefly some patterns in animal behaviour that are probably related to the distribution of secondary compounds in plants, and outline some hypotheses in chemical ecology which that behaviour suggests. I will focus on those hypotheses that the ecologist has little chance of examining on his own, but seem imminently approachable by the chemist working with the ecologist.

THE ANIMALS

We could use almost any large group of phytophagous insects or verte-brates to generate these hypotheses. I have chosen pea weevils, small beetles in the family Bruchidae, because I am familiar with their biology and am currently conducting a long term study of their host-specificity on a com-munity-wide basis (in a tropical deciduous forest, Guanacaste Province, Pacific coastal lowlands of Costa Rica, Central America). Their basic biology is as follows (and see Janzen[3, 4, 12–14] and included references). The female beetle spends about 6 to 9 months of the year as a reproductively inactive adult living among the vegetation. When her host plant produces a seed crop, she moves to the plant and lays eggs on the seeds or pods (fruits). The larvae bore into the seed (or eat the entire seed) and mature there, feeding on the seed contents. After a short pupal stage, the adult emerges from the seed. If there are still susceptible seeds available, there may be a second generation of beetles. In the field, host-specificity of the beetles is determined by collecting large samples from seed or fruit crops, allowing the adults to emerge into sealed containers, and later identifying them.

BRUCHID HOST-SPECIFICITY

Tropical bruchid beetles are undoubtedly among the most specialized and host-specific of insects. To date, I have reared at least 101 species of bruchids from seeds and fruits collected in the deciduous forests of Guanacaste; of these beetles, 94 have only a single (but different) host plant. The remaining 7 species have only 2 host plants each. This amazing specificity occurs in a forested region that contains at least 1000 species of seed-bearing plants. More than half the bruchid hosts can be found in any one site of only a few square kilometres, and most of the hosts bear seeds at the same time of year (the dry season). The seed crops of almost all potential hosts have been examined during the past two years. This is the first tropical community where a study has determined with certainty that not only does insect species X_i feed on plant species Y, but also species X_i does *not* feed on plant species $A \ldots X$ and Z.

I feel certain that this high degree of specialization is determined largely by the nature and concentration of secondary compounds in the seeds rather than the seeds' nutritional values. To a lesser degree, the pattern of host specificity is probably determined by fruit morphology, phenology and secondary compound chemistry. While we need much more direct evidence from the chemist to deal definitively with the subject, several indirect lines of evidence support the hypothesis that the secondary compound chemistry is important (and studies are under way to increase the evidence). The facts so far found are:

(a) When eggs are laid on seeds other than those of the usual host, the larvae bore into the foreign seeds, but then die almost immediately after chewing into the cotyledons.

(b) In the laboratory, one species of bruchid may develop in the seeds of many species of domesticated (commercial) beans. The seeds of these species of domesticated beans have one outstanding trait. They have had the alkaloids or uncommon amino acids, that are found in their wild relatives, bred out of them (or perhaps these species were initially selected for cultivation because their seeds were not toxic).

(c) While many of the seeds attacked by bruchids are not noted for extreme toxicity to other animals, the unattacked species are often noted for containing high concentrations (e.g., 5 to 10 per cent dry weight) of potentially toxic compounds (e.g., *Canavalia* with canavanine (I), *Mucuna* with L-DOPA (II), *Enterolobium* with albizziine (III), *Erythrina* with erysodine (IV), *Guilandina* with γ-methylglutamic acid (V), *Schizolobium* with schizolobic acid (VI), etc.)[7, 8, 12, 15]. It is not surprising, however, that some seeds eaten by bruchids are poisonous to other animals; the bruchid has simply become a specialist at detoxifying or otherwise avoiding the secondary compound.
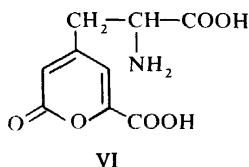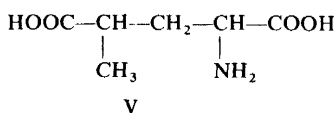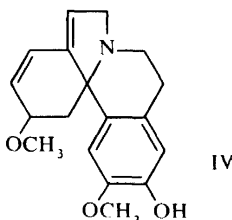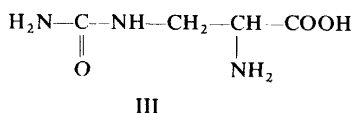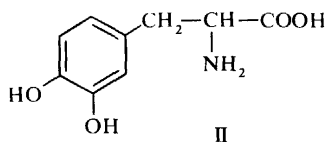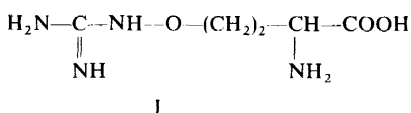
At this point we need one additional piece of background information. There is no evidence that the female bruchid is repelled by the secondary compounds within the seed, as if they made the seed directly distasteful or repulsive. Her behaviour indicates that she recognizes only the reproductive structures of her proper host plant as oviposition sites. For example, if a female *Mimosestes sallaei* is placed in a bottle with seeds of her proper host (*Acacia farnesiana*) and seeds of the species mentioned in the previous paragraph, she will oviposit on all of them, but her larvae will die after feeding. Were a mutant strain of *A. farnesiana* to arise that had seeds that differed only by containing a compound that was toxic to the beetle, she undoubtedly would continue to oviposit on it until natural selection removed her genotype from the bruchid population. Likewise, mutant strains of beetles that will oviposit on seeds of plants other than the usual host must occasionally appear during evolutionary time; they must perform this novel oviposition while having no awareness of whether the new host will be toxic.

As an ecologist, I can work out the fidelity of the bruchids under natural and laboratory circumstances. However, a number of questions come to mind that cannot be answered without extensive help from the chemist.

IS THERE CHARACTER DISPLACEMENT IN SEED CHEMISTRY?

Character displacement is probably a common phenomenon between animal populations, though very difficult to demonstrate[16]. As generally viewed, the phenomenon is that a pair of similar species with partly over-lapping ranges are more dissimilar in the area of overlap (in respect to some trait related to the way they compete) than in the regions where they do not co-occur. The analogous phenomenon with the bruchid–seed interaction may be expressed at two levels of community organization. (a) A pair of related plant species may have different secondary compounds in their seeds in their area of overlap while having similar defensive compounds in regions where they do not co-occur. Such geographic variation will be difficult to detect as long as analysis of seed chemicals is done without regard to seed origin with respect to the other species in the habitat from which the seed is drawn. (b) At higher levels of community structure, we might expect the interspecific richness of secondary compounds in seeds to be considerably greater within a given habitat than between habitats. The most extreme example of this would be a case where each habitat has an array of the same secondary compounds, but in each habitat a given secondary compound is found in the seeds of a different species of plant. The ecological–evolutionary processes that could generate such patterns in the secondary compounds in seeds are relatively simple in concept.

Let us first examine the case of character displacement with two similar species of plants that initially have the same chemical defence for their seeds and are therefore attacked by the same species of specialized bruchid. Here, there should be an exceptionally high probability that a resistant mutant strain of one plant will be favoured in the geographic region of overlap. This should be so for two reasons. First, there is a reduced likelihood that a new resistant strain of bruchid that can counter the new plant defence will

appear; this is because the beetle will still have at least one host in the habitat and therefore not be forced to extinction or to change hosts if a resistant phenotype fails to spread. Second, with two plants with the same defence in the same habitat, the bruchid that is resistant to that defence will be able to build up to higher population densities on the average than it could on one host alone (and therefore cause higher percentage seed destruction). This means that there will be stronger selection favouring a mutant strain of plant in either of the plant species than would be the case if the defence were confined to one plant.

When we take into consideration the entire array of plant species in one habitat, there will be the processes described above for each pair-wise combination of species, plus the following processes. As the number of species using the same defence in one habitat increases over evolutionary time (through mutation and immigration), the probability that an insect (or additional insects) will breach this defence greatly increases. This is so for two reasons. First, the more plant species in a habitat that have a given defence, the more likely it is that at least one will resemble (on other traits) the host plant of some seed-eating insect that is being forced to change hosts or is capable of expanding its resource base. Second, in those cases where the insect (or other animal) can feed on several species of plants, the more the species using the same chemical defence, the larger will be the resource base available to a new mutant strain of animal that has breached that defence.

Let us examine an example from the Costa Rican tropical deciduous forest that was mentioned earlier. In this forest there is a common vine (*Dioclea megacarpa*, Leguminosae) with seeds that contain 6 to 11 per cent dry weight of the generally toxic uncommon amino acid, canavanine (I)[7]. These seeds are fed on by the larvae of the bruchid *Caryedes brasiliensis*, which is host-specific to this plant in this forest[4]. In the same forest there is another vine (*Canavalia* sp., Leguminosae) with large seeds (as are those of *D. megacarpa*) that also contain large amounts of canavanine.† This species of *Canavalia* is not at present attacked by any bruchid. However, its chances of being attacked by a bruchid in the evolutionary future should be much greater on the average than are the chances of plant species that lack a defence mechanism in common with other plants that are attacked by bruchids. For example, *Mucuna andreana* (a vine in the same forest area) has seeds that are 5 to 10 per cent dry weight L-DOPA (II)[8, 15] but superficially so similar to *Dioclea* that taxonomists often confuse them. It is much less likely to be attacked in the future by *Caryedes brasiliensis* than is *Canavalia*. This hypothesis is supported by two arguments. First, the presence of *Car. brasiliensis* means that *Canavalia* has a known potential seed predator. Second, the presence of *Car. brasiliensis* demonstrates that a canavanine-resistant bruchid can exist, whereas there are no known examples of L-DOPA-resistant bruchids. Incidentally, I may also note in conclusion that if the bruchid should shift onto *Canavalia*, the density of this vine may change, it may disappear from the habitat, or its chemical defence system may change. Either of the latter two cases would increase the species richness of secondary

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† E. A. Bell, personal communication.

compounds in the habitat, provided that the out-going *Canavalia* was replaced by an immigrant species of plant with a different defensive compound.

A few words may be appropriate here on the quirks of host-switching by host-specific herbivorous insects. Evolutionists are quick to note that once the bruchid begins to feed on a new host (as the result of new resistance in the old host), the selective pressure against the susceptible strain of the original host is relieved and therefore the resistant strain of the original host will no longer be favoured. This is not a serious problem, as suggested by the following considerations. There is very high survivorship of the bruchids on their original susceptible host; on a new resistant strain there is likely to be little or no survival. This great difference in survival will greatly increase the relative value of an alternative host that is normally suboptimal. There should be considerable time before the bruchid has evolved until it is doing as well on its new host species as it did on the old. During this time, the bruchid population should maintain continual selective pressure against the susceptible strain of the original host, thereby insuring replacement of the susceptible strain by the strain resistant to the bruchid.

How does one test for character displacement at the level of seed chemistry? As suggested earlier, pair-wise testing simply requires the mapping of chemical diversity with respect to the overall occurrence of secondary compounds in the habitat. However, there is another and perhaps more interesting test.

If character displacement is operating, we should expect greater chemical similarity among the plant parts eaten by one herbivore guild than between the various parts of one plant. This is due to the patterns of specialization shown by herbivores. For example, the insects that feed on seeds do not usually feed also on leaves or roots of their host plant, and the root-feeding guild is generally not found eating flowers or stems. The result is that the same chemical defence may appear in different plant parts of one species without increasing the probability of any one part being attacked. It should be noted that an upper limit to such a loss in chemical diversity should be reached when the amount of plant biomass protected by one compound becomes high enough for a large animal to specialize on it. A large animal could conceivably have a sufficient complex behaviour and morphology to harvest from all the plant part subsets of the habitat. It is somewhat surprising that we do not have a large mammal that is specialized at feeding on all parts of the tannin-rich tropical mangrove forest. In summary, if character displacement is operating within one type of plant part, then we may expect a great deal of redundancy in chemical defence when pooling the sets of plant parts fed on by distinct herbivore guilds within a habitat. Unfortunately, we may also expect this result because once a plant has the biochemical machinery to make one defensive compound (e.g. in seeds) then there will be selection favouring its incorporation in some newly attacked part (e.g. roots) rather than the evolution of a quite new compound, simply on the basis of economics.

It must be added that high intra-habitat species richness of secondary compounds, and low inter-habitat species richness of secondary compounds, can be achieved through two related processes. As described above, character displacement can occur *in situ* during the evolution of chemical resistance.

On the other hand, the presence of one chemical defence, already breached by one specialist insect, can result in an invading plant species that uses the same chemical defence failing to become established (owing to being attacked by the bruchid already present). Alternatively, the invader may cause the removal of the resident plant species. This might happen if the invader is less influenced by the bruchid than is the resident, yet is an alternative host for the bruchid to increase on resulting in more severe damage to the resident species than before.

HOW MUCH CHANGE IN SEED CHEMISTRY IS REQUIRED TO REMOVE A SEED PREDATOR?

It was implicit in the previous discussion that over evolutionary time, the bruchids rotate among the host plants within a habitat (and more rarely, between habitats). We may expect such rotations to most commonly occur when a mutant strain of plant appears that is resistant to the usual bruchid for that plant. The mutant strain of plant must be as or more dissimilar from the parental phenotype as the parental type is from the other plants in the habitat. If this were not so, the bruchid would most likely move into the resistant strain rather than change host species. We have, however, a large problem in understanding chemical dissimilarity as used here and in previous sections. From the bruchid's viewpoint, such dissimilarity can only be measured in units of relative reproductive success on the various possible host species as contrasted with the mutant strain. Determining this is a task for the ecologist. However, from the viewpoint of the chemical ecologist, there may be the possibility of correlating the change in seed chemistry with the change in fitness of the bruchid. The question then becomes, how do we qualify chemical dissimilarity so that it will be correlated with what the bruchid regards as dissimilar?

One approach (but probably impractical) is to measure the average number of mutations required to modify a toxic secondary compound that can be detoxified by a specialist bruchid into a secondary compound that will kill the bruchid. The serious practical problems notwithstanding, such understanding is badly needed in order to understand the degree of evolutionary conservatism in the animal–plant–secondary-compound interaction. I suspect that most resistant plant phenotypes initially differ from their susceptible parents by only a single gene. In addition to the genetic difficulties, there are also three major ecological problems with such a measure of dissimilarity of chemical phenotype.

(a) Many toxic secondary compounds display dosage effects. The parents of a toxic seed may differ from those of a palatable one only in the number of identical loci associated with the trait. As Levin[6] and Whittaker and Feeny[10] have stressed, polyploids may produce much higher concentrations of secondary compounds than their parents.

(b) Irrespective of dosage effects, many secondary compounds are only sub-lethal in that they do such things as slow the larval development rate, lower the eventual adult fecundity, etc. Changes in such sub-lethal consequences will be extremely difficult to monitor following single-gene changes in chemical phenotype.

(c) There are dramatic differences in susceptibility to secondary compounds among the various major groups of herbivores. Thus a fungus, such as the one cultivated by neotropical leaf-cutting ants, may be able to deal with the secondary compounds in the leaves of many tens of species of very different dicotyledonous plants. Similarly, ruminants, by virtue of their intestinal microflora, seem to be able to consume small amounts of many secondary compounds that are toxic in higher concentrations. Insects, on the other hand, and especially the host-specific ones, seem to be able to deal with the secondary compounds of one or a few plant species at the most.

There is another approach that is probably more practical from the chemist's viewpoint. Here, we would measure the average chemical dissimilarity between the secondary compounds to be found, for example, in the seeds of a given habitat. This is essentially a taxonomic problem, and assumes that the average chemical dissimilarity is a good measure of the amount recognized by the bruchid as sufficiently great to maintain high host-specificity.

For example, the seeds in a tropical dry forest may contain such compounds as canavanine (I), L-DOPA (II), albizziine (II), erysodine (IV), γ-methylglutamic acid (V) and schizolobic acid (VI). If we take the average dissimilarity among such compounds to be that which is necessary to generate high host-specificity (against this particular ecological background of host-plant community structure), then in what units shall we measure dissimilarity? Obvious candidates are such things as molecular weight, number of methyl groups, number of points of attachment to a substrate, number of atoms difference between them and the molecules that they mimic (e.g. canavanine–arginine), etc. This seems a place for some form of numerical taxonomy whereby large numbers of traits would be taken into consideration in constructing similarity matrices for the secondary compounds.

There seem to be two choices in such a taxonomic operation. We may choose molecular characteristics haphazardly ('at random') and then with the data for a large number of characters in hand, calculate similarity indices by computer. On the other hand, we may well operate as would the classical taxonomist. In this case, we could guess at which molecular characteristics should impart toxicity, and restrict our measurements to those traits. The classical taxonomist turns to the fossil record and Darwinian inference to bolster his intuition: we would have to turn to the biochemist and pharmacologist to bolster ours.

This brings to mind the possibility of deriving measures of overall toxicity of secondary compounds in the context of the entire animal as a community of chemical reactions. For example, it appears that many uncommon amino acids are toxic because they are analogues of the amino acids normally incorporated in proteins. In any particular tissue system, each protein amino acid will have a different relative importance to the animal; some amino acid substitutions would be more disruptive than others in protein synthesis. It may also be that when this disruptiveness is summed over the entire animal, some protein amino acid substitutions will on the average be more disruptive than others. If we then score the 100-plus species of uncommon amino acids with respect to which protein amino acids they

mimic, perhaps we would find that some protein amino acids have many more mimics than others. The prediction then becomes that the most important protein amino acids should have the largest number of uncommon amino acid mimics. In other words, the results of many episodes between insects and seeds over evolutionary time should be that on the average those animal biochemical systems most badly damaged by amino acid mimics should have been most commonly the 'targets' in the evolution of chemical defence by the plant. The primary sources of variation in such a system should be the ease with which certain species of uncommon amino acids can be derived evolutionarily, their cost in biosynthesis, the ease with which a protein amino acid can be mimicked, and their ease of detoxification by the animal.

WHAT IS THE SECONDARY COMPOUND BATTLE-PLAN OF AN ENTIRE PLANT?

It is clear from this discussion that we need two quite different kinds of chemical blueprints at the community or habitat level. We need surveys of the secondary compound defence mechanisms for all the seeds, roots, or shoot tips, etc. for a particular habitat. This should not be restricted to one class of secondary compounds. Ideally, it should be supported by estimates of concentrations and their variations within and between plant species. On the other hand, we need detailed maps of the secondary compounds within some entire plants: maps that take into account different groups of secondary compounds and their detailed location in the plant. I have been speaking as though a bite of a plant contained only one defensive compound. More than likely, it contains many (and the possible synergistic effects are immense). It will be a huge task to gather this information, and there may be several ways to lessen the load.

(a) Rather than try to tackle the problem in many habitats or communities at once, it is probably best at this stage to choose one complex community and put much effort into it.

(b) Representative plants could be chosen for examples of the total plant blueprint asked for above, but how to decide which are representative is most difficult.

(c) To know what our plants contain, we could rely on chemical surveys done on the same 'morphospecies' of plants as occur in our chosen habitat but growing in different parts of their ranges. This, however, has the outstanding problem that we know almost nothing of geographic heterogeneity in secondary compounds for particular plant parts in complex tropical communities. For example, it appears that we have no right to infer that *Enterolobium cyclocarpum* has albizziine (III) in its seeds all the way from Mexico (where it is not attacked by bruchids) to Brazil (where it is attacked by a bruchid).

(d) Plants from the main study site can be grown in greenhouses and gardens near the chemist's laboratory. This removes the problem of decomposition of secondary compounds during preservation of the plant in the field, but it adds the important problem that we do not know whether defensive compounds are produced in their normal concentrations (if at all) under greenhouse conditions.

In conclusion, it appears that we should be arguing for the development of chemical ecologists who are effectively 'general practitioners' of secondary compounds (as well as for the development of a more precise terminology than 'secondary compounds'). Such a scientist would specialize in identification and functional significance of secondary compounds, irrespective of their chemical species. Clearly this person would also be a specialist at knowing to whom to send compounds for analysis. If I may reason from my own experiences as an ecologist ('jack-of-all-trades, master of none'), such a scientist will initially be faced with the huge problem of lack of respect by his more specialized peers. We may be able to do more for the art of understanding the chemistry of animal–plant interactions by providing a psychologically pleasant atmosphere for the chemist in this area than by increasing his funding or by technical advances.

ACKNOWLEDGEMENTS

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