Why Do Organisms Make NPs?

Richard Firn

DOI:10.1093/acprof:oso/9780199566839.003.0005

Abstract and Keywords
This chapter discusses why organisms make Natural Products. The most widely accepted model, the Chemical Co-evolution Model, proposed that the interactions between a plant species and insects (which interact positively or negatively with the plant) were shaped by NPs made by the plant. For example, a plant making a novel NP would gain fitness if that NP reduced the fitness of herbivores, or the plant would gain fitness if the NP had a beneficial effect on insects whose visits benefited the plant. The evolutionary response of the insects would be to adapt to these new selection pressures and this would result in the insects becoming increasingly specialist. The Chemical Co-evolution Model was based on the assumption that every NP possessed (or had possessed at some stage in evolution) some biological activity that enhanced the fitness of the producer. However, this assumption is not supported by experimental evidence, and the assumption has no theoretical basis. The Screening Hypothesis seeks to explain the evolution of NPs when the chances of any one NP benefiting the producer are indeed very low. This simple hypothesis predicts that certain metabolic traits which favour the generation and retention of NP diversity will be retained during the evolution of NP metabolism. The most important of these predicted metabolic traits is the ability of enzymes making NPs to either accept more than one substrate or to make more than one product. Evidence consistent with the Screening Hypothesis has grown in the past decade such that it is now a credible model based on sound physicochemical and evolutionary principles.

**Keywords:** reductionism, Natural Products, NP metabolism, Chemical Co-evolution Model, Screening Hypothesis, evolution

*In some scientific circles it is something of a sport to theorize about function, often with the intent of finding one overriding axiom true for all secondary metabolism. Speculations range from the notion that they are waste products or laboratory artefacts, to the concept that they are neutral participants in an evolutionary game, to ideas of chemical weaponry and signalling.*

—Bennett, 1995
Summary
There have been many attempts to explain why organisms make NPs. The most widely accepted model, the Chemical Co-evolution Model, proposed that the interactions between a plant species and insects (which interact positively or negatively with the plant) were shaped by NPs made by the plant. For example, a plant making a novel NP would gain fitness if that NP reduced the fitness of herbivores or the plant would gain fitness if the NP had a beneficial effect on insects whose visits benefited the plant. The evolutionary response of the insects would be to adapt to these new selection pressures and this would result in the insects becoming increasingly specialist. This model argued that the great diversity of NP structures resulted from the great diversity of the co-evolutionary processes in the natural world. The model was easily applied to plant-fungal interactions but was least convincing when explaining the more ancient NP diversity in microbes.

The Chemical Co-evolution Model was based on the assumption that every NP possessed (or had possessed at some stage in evolution) some biological activity that enhanced the fitness of the producer. This assumption is not supported by experimental evidence and the assumption has no theoretical basis. Extensive studies of collections of both synthetic chemicals and NPs have shown that the probability of any one chemical structure possessing potent, specific biological activity is very low. These experimental findings are supported by the current understanding of the way in which small molecules interact with proteins to bring about biomolecular activity.

The Screening Hypothesis seeks to explain the evolution of NPs when the chances of any one NP benefiting the producer are indeed very low. This simple hypothesis predicts that certain metabolic traits which favour the generation and retention of NP diversity will be retained during the evolution of NP metabolism. The most important of these predicted metabolic traits is the ability of enzymes making NPs to either accept more than one substrate or to make more than one product. Evidence consistent with the Screening Hypothesis has grown in the past decade such that it is now a
credible model based on sound physicochemical and evolutionary principles.

Some criticisms of the Screening Hypothesis helped refine the model and the thinking behind the model has since been applied to a wider range of metabolism (see Chapter 9).
Reductionism—a scientific tool only as good as its user

Our understanding of the natural world comes from identifying, classifying and determining the function of its components. The process of gaining information about the natural world has progressed at a rate dependent partly on what was technically possible to those carrying out the studies and partly on the state of intellectual development. One of the most successful approaches to carrying out scientific studies has been reductionism. The investigators attempt to identify the key components that make up a large, complex process. They then study the individual parts in isolation, trying to understand what each part does. Once the role of every part is established, a view of the overall process can be formed by assembling all the individual ideas in a coherent, logical manner. A good analogy of the benefit of studying such complex objects in this way would be the study of a car. Most people can grasp the concept of what a car is capable of doing but trying to understand how a car works requires the attention that is paid not to the whole object but to the individual parts. The whole object rolls back or forward because it has wheels. The object can move in different directions because the front wheels pivot. The force to turn the driving wheels comes via gears and shafts from another complex item that will function in isolation—the engine. However, when using a reductionist approach, caution is needed in choosing the appropriate level of scale for the analysis. Taking a car completely apart, to the final screw and spring, without understanding the purpose of the component from which the screw or spring is being removed starts to reduce the quality of information being accumulated. One would have little chance of understanding what a car was if one only had a huge heap of individual parts not organised in any way. So, reductionism is a powerful tool, but only if used wisely.

Using the car analogy to illustrate the power and problems of reductionism should not encourage the view that biological systems are simply like machines. One key difference is that ‘the evolution’ of the car and the evolution of an organism are very different processes. Humans in their endeavours can be informed by experience and knowledge but at any time humans can introduce radical changes—a new design can start with a clean sheet of paper and a new design can be radically different from anything made before (e.g., front wheel drive...
Why Do Organisms Make NPs?

vs. rear wheel drive). In human artefacts, there is ‘an evolution’ of thought in the design of the object but the manufactured object can be unrelated to any previously made object. In contrast, biological evolution always starts with an existing system, then many minor variants are made which are based entirely on (p.93) the original design and each variant is allowed to compete against the original and all other variants. If a variant is made that performs better than its competitors then it will have a better chance of reproducing and its genes, which code for the new variant, will increase in frequency in the population.

Reductionism has been very evident in studies of NPs. The study of NPs passed quickly from herbalism to the study of chemicals at a time when evolutionary thinking was only just developing. Because those chemical studies were taking place in Chemistry Departments (see Chapter 1), the reductionist approach tended to concentrate on characterisation of yet more and more chemicals. Biochemists, universally more schooled in chemistry than evolutionary theory, tended to focus on the role of their favourite enzymes in making one type of NP—the normal reductionist approach adopted by biochemists. When biologists began to participate in exploring NPs, each biologist tended to study one particular NP (or maybe a related family of NPs) and very often they concentrated their thoughts on one process in their chosen model organism. Thus, those working on NPs were not only fragmented in different scientific disciplines but also each had, rather too rapidly, reached a very high level of reductionism (the analogy of the car being stripped to individual components before the main functional parts were understood comes to mind). While the reductionist approach was successful in each area, too few people stopped to ask the question as to why organisms had an ability to produce all these wonderful, striking chemicals. By the middle of the twentieth century, there was no shortage of information about the tens of thousands of individual chemical structures (published in specialised chemistry journals), about the biochemical pathways that form each major class of NP (published in biochemistry journals), about the properties of some enzymes in these pathways (sometimes published in even more specialised biochemistry journals) and about the properties of some NPs that had notable effects (often published in specialised medical journals or cell biology...
Towards the end of the twentieth century, this fragmentation actually increased as more biologists took an interest in NPs but they tended to publish their work in even more specialist journals, journals often targeted at even smaller groups. However, in this chapter, we shall leave the wealth of detail in the background and try to show how the few people thinking about why organisms make NPs were developing their ideas.

The development of ideas about why organisms evolved to make NPs
Nineteenth-century scientific work on NPs

From the fifteenth century onwards, the major European powers were sending plant collectors to scour the world for new, exotic plants and the number of species that were accessible to herbalists and physicians increased. The great universities began to assemble plant collections in Botanical Gardens and some royal collections were also established, the best known perhaps being Kew Gardens in London. The reasons (p.94) for gathering such collections varied. Some scientists desired to have access to a wide range of fascinating biological specimens in order to try to understand the natural world in all its diversity. Others wanted to ensure that they had access to specimens of all plants that may have commercial value so they could be introduced into new colonies (as outlined in Chapter 2, high value plants were often jealously guarded to maintain a monopoly of supply). However, some plant collectors had neither scientific nor commercial motives, rather they were simply obsessed by the possession of rare specimens (although as the Dutch tulip mania had demonstrated in the seventeenth century, rarity can get translated into commercial gains for some but ruin for others).

Of course, there was a widely held view in nineteenth-century Christian Europe that God had created all organisms for man's benefit (it was man's benefit), hence many considered that the existence of NPs as requiring no explanation. However, some scientists began to see the need to seek explanations of how the natural world had been formed. A common theme began to emerge—the natural world was not a fixed entity that had been created as it now existed, rather the world as we experience it at any time is changing due to the action of various natural forces. Geologists, led by the Scot James Hutton, speculated how mountains arose, why great valleys had been formed and why soils varied. Biologists, building on the work of the Swede Carl Linneaus, had begun to understand the relationship between different organisms. After Wallace and Darwin had outlined the principle of natural selection, it was soon widely accepted that individual organisms were subject to competitive selection. An individual making something, which has a significant cost of manufacture or maintenance, that brought no increase in fitness would be less fit than an individual that lacks this redundancy. This principle would apply even at the chemical level. However, as
introduced in Chapter 1, the study of NPs during Darwin's
time was largely a matter of determining the structure of
individual NPs and trying to find ways of making them
synthetically; this work was carried out almost exclusively by
chemists who were largely uninterested in why organisms
made these fascinating structures. That set a pattern that was
to continue for a century, where the study of NPs as chemicals
was divorced from the study of the organisms that made them.
Twentieth-century scientific work on NPs

Throughout much of the twentieth century, chemists continued accumulating information on the structure, and sometimes the synthesis, of individual NP chemicals. A massive literature grew about all the weird and wonderful chemicals. These wonderful structures challenged and fascinated generations of NP chemists. Even after biochemistry departments began to appear, the study of NPs largely remained in chemistry departments, because biochemistry departments were heavily biased towards the study of the major anabolic and catabolic pathways found in most groups of major organisms. Journals that specialised in the chemistry of NPs were published long before journals appeared that asked why organisms made them.

(p.95) Despite the general lack of curiosity of why organisms might be making these important molecules, a very few individuals in the first half of the twentieth century did begin to explore various ideas as to why some organisms (it was now clear that microbes as well as plants made NPs) made NPs. However, the diversity of NP structures made it hard for anyone to come up with a universal explanation. This lack of a universal explanation was not of great concern because the increasing importance of NPs as pharmaceutical products in the second half of the twentieth century (led by the discovery of penicillin as outlined in Chapter 7) gave NPs an importance beyond whatever role they played in the organisms that made them. The huge new economic importance of the antibiotic NPs stimulated a demand for NP chemists. The other academic discipline which gained from this new interest in NPs was microbiology. Microbiology departments had flourished in the early twentieth century because many common diseases were caused by microbial infection and the ability to identify microbial contamination of food and water offered the potential to improve public health. Although chemists were the first to offer chemicals to attack pathogen organisms, after the discovery of microbial antibiotics, the selectivity and potency of these agents gave microbiologists a new role in helping fight infectious diseases. However, interest in NPs in the more mainstream biology disciplines, such as botany or zoology, remained small. This only changed a little in the third quarter of the twentieth century but by the last quarter, the study of the role of NPs had become respectable for a wide range of biologists. This was helped by the fact that
modern agriculture was fighting a constant battle with crop pests and diseases; attempts to understand the natural defences of plants, postulated to involve NPs, was timely.

As noted in the quotation at the beginning of this chapter, towards the end of the twentieth century, some scientists even played down the importance of asking why NPs were made by organisms and seemed unembarrassed by the failure of those studying NPs to have a convincing universal model. As is sometimes the case when no universal model for a phenomenon is widely accepted, the problem is not a lack of theories rather there are too many. The problem was that none of the theories seemed to explain all the NP diversity that chemists continue to find.

Several ideas were advanced for why organisms made NPs

Waste products

Animal physiologists had long worked out that animals had evolved sophisticated structures in order to deal with waste products. Thus, it somehow entered the thinking of some biologists that all organisms would have to have some equivalent mechanism to get rid of waste. Because plants and microbes clearly did not have livers or kidneys, but they did have all these weird chemicals, it was proposed that these two facts were related. Maybe plants and microbes had no choice but to make weird chemicals in order to get rid of some other chemicals that were troublesome for them? Consequently, NPs were just waste products. What is so remarkable with this idea (p.96) is that it was ever taken seriously. It shows such a complete misunderstanding of plants and microbes.

Why do animals produce waste? Each animal species has evolved to be able to survive on its own unique diet. However, all the different animal diets have one thing in common—they are made up of a complex mixture of complex chemicals and in many cases the diet will change with time. Animals vary in their ability to cope with a varied diet. Some species have evolved to be specialists, living on a very limited range of hosts (e.g., aphids). However, many other animals are generalist. Generalists have to survive on whatever they can find in their vicinity and have to be adaptable. In all cases, however, seeking the perfect food source—one that contains exactly the elements needed, in the right proportion and nothing else—is a risky strategy so evolution has favoured animals either have a digestion
system that is versatile enough to take nutrition from various food sources\(^3\) or an excretion system that can rid the body of all the unwanted chemicals ingested or created by the digestive processes. Consequently, even though each species may have some ability to select its food intake, there will be an inevitable mismatch between the chemicals needed to sustain the animals and the chemicals in the diet. In order to ingest the chemicals needed for sustenance, some unwanted chemicals must be taken in by the animal. In the extreme case of a generalist, on some days there may be too much protein yet on another day there may be too much carbohydrate or fat. On many days some chemicals will be ingested that simply cannot be assimilated. Consequently, the digestive system of each species has evolved to remove what that species needs from its mixture of ingested chemicals and to excrete the remainder as waste. The fact that one species of animals (e.g., a dung beetle) may gain its nutrition from the waste of another species (a cow) illustrates the fact that the term ‘waste product’ is a relative term.\(^4\)

**Why should plants produce waste?** Plants and microbes, unlike animals, do not ingest chemically complex materials in order to gain nutrients. In contrast to animals, plants take in a very few simple molecules (water, carbon dioxide, nitrate, phosphate, other ions, etc.) which they use to elaborate more complex molecules. The plants have evolved to control the uptake of many of these elements, including all those elements used to make NPs. The plant with access to surplus carbon (available in infinite amounts in the air as carbon dioxide) can reduce the rate of photosynthesis or can store any surplus carbon in starch for example. A plant that suddenly finds its roots exploring a nitrogen-rich part of the soil (maybe where a worm is decaying) need not be stressed by the now unbalanced supply of nitrogen relative to carbon because the roots could take up less nitrogen, or more likely the surplus nitrogen could be taken up by the plant and stored in a storage protein. Likewise, for nearly every element, the plant has some control of the rate of uptake and usually has a route to an appropriate storage compound. Clearly, plants, in complete contrast to animals, have evolved to absorb a simple, predictable mixture of chemicals and the concept of waste is largely inappropriate. Furthermore, when thinking about the possibility that NPs
were waste products, why would each (p.97) plant species have evolved a novel NP composition given that they and their ancestors would have taken in the same mixture of simple molecules. In other words, the proposal that NPs are waste products is based on a false premise (that plants produce waste) and cannot even explain NP diversity.

Why should microbes produce waste? Many microbes, like plants, have considerable control over their intake of simple substances. Although many microbes do use complex molecules as a source of necessary elements, microbes commonly excrete degradative enzymes that breakdown the complex molecules into simpler substances, substances that can be taken up by the microbe in a controlled way. A characteristic of many microbes is their ability to alter their immediate environment and that includes their chemical environment. However, because of the diversity of microbes and their remarkably versatile biochemistry, one can never dismiss the possibility that some NPs made by microbes could serve a minor role in ‘waste management’.

NPs are test chemicals made by ‘Inventive Metabolism’

Given that natural selection operates by selecting the fittest from all the variants in a population, a mechanism must exist to generate the variation needed. Mutations causing small changes in the base sequence in the genome are the main source of this variation. It is easy for biologists to see the result of this variation if the changed phenotype is evident at the level of the whole organism. Such morphological variation, for example, was evident to those selecting features in domesticated plants and animals for thousands of years. However, biochemical variation is less easy to perceive because, unless it is manifested by a large visible change (e.g., flower colour, leaf variegation or fruit colour), the changes are hard to detect. Furthermore, the concentration of individual chemicals sometimes changes continuously on a daily and seasonal basis and as such it is hard to evaluate the basis of some biochemical changes, even if one can measure them. Because of homeostatic controls that also operate to maintain biochemical concentrations within certain tolerable limits, a mutation causing a change in the capacity of the cell to make a certain chemical might only be evident under certain specific conditions. However, the selection of variants from populations of plants show that even for NPs, individuals differ, sometimes remarkably, in their NP composition. The variation can be of
two kinds. The relative amounts of each of the many NPs being made by species may differ. For example, a mutant plant that smells different from all others might only have increased the concentration of an existing compound to a level that brings it into the range that the human nose can detect. This kind of variation is relatively simple to explain in terms of a mutation having an effect on a pre-existing control process. However, more challenging is to explain how mutations produce new chemical entities. Zähner, discussing how microbes might produce new chemical diversity, proposed that NPs were the route to new chemical structures. He proposed that the metabolism that generated NPs was retained by microbes in order to retain a capacity for what he called biochemical inventiveness. This imaginative idea seems to have been a minority view (p.98) but it freed any individual NP from having a particular role, assigning a role instead to the overall metabolic capacity. One major problem with the model in evolutionary terms was why so much inventiveness was retained in a population. It would normally be expected that any mutant that possessed a novel, but useless, biochemical capacity would be lost from the population because the cost of making useless chemicals would make that individual less fit. Thus, the inventiveness would be expected to exist more at the level of the individual rather than the population. Although individuals in a microbial population might be predicted by Zähner's model to possess different NP compositions, the model could not easily explain why many individuals in a population of one plant species would all possess such a rich and characteristic NP composition simply to retain this inventiveness. Another challenge to Zähner's hypothesis was that it did not adequately define for what purpose the biochemical inventiveness was retained. How would throwing up new chemical structures enhance the fitness of the producer? More specifically, how could any of the new structures generated by such biochemical inventiveness integrate into the main metabolic functions of the organism in a way that would enhance fitness? The structures of most NPs are very different from the chemicals that are universally made by most living organisms.

NPs are relics of previously important cell regulators

Another microbiologist, Julian Davies, was struck by the fact that some NPs found in microbes had powerful inhibitory effects on the synthesis of some very important pathways. He
argued that some of the powerful antibiotics found in microbes were relics of regulatory molecules that had once been used by microbes to regulate their own biosynthetic activities. The basis of this proposal was that some antibiotics are so potent and specific in their ability to inhibit very basic mechanisms in the other microbial cells that they must have, at some time, evolved to act on those specific inhibitory sites. The main problem with this model is that it cannot account for a very large number of NPs that have very low biological activity and no known antibiotic activity. Davies’s theory is too focused on antibiotics to be a general explanation for the existence of all NPs. The fact that some antibiotics have these poisonous effects on some cells is actually a very biased piece of information because antibiotics have been selected by humans from thousands of NPs found in microbes because they possess unusual properties. Hence, these unusual properties are unlikely to offer much help in understanding why the microbes that make them also make many more chemicals with no great potency as antibiotics. At best, the model could account for only a very minute fraction of NP chemical diversity, hence is unattractive as a general model to explain NP diversity.

**NPs are made for no reason—they are fortuitous**

As the twentieth century progressed, a number of microbiologists found that all the explanations advanced for why microbes made NPs were unconvincing when applied to their microbial NPs. Hence, many simply accepted that microbes made all sorts of weird chemicals because the costs associated with their production were so low that their production was not selected against. Evolutionary biologists were always very sceptical about such arguments but not all microbiologists interested in NPs were troubled by the views of evolutionary biologists. Furthermore, a microbiologist interested in finding new antibiotics often felt that evolutionary arguments were not going to help them in their quest.
NPs serve many roles in influencing the species-species interactions—the Chemical Co-evolution Model

One of the biggest problems that scientists face when trying to devise a theory to explain some piece of complex biology is knowing which pieces of information they should use as the building blocks of their model. By the last quarter of the twentieth century, the amount of information published about NPs was vast. The chemical structures of tens of thousands of NPs had been reported; the biological properties of many of these had been studied (at least to the extent that they had been passed through some drug screening trial by a pharmaceutical company or a government agency); thousands of papers had been published about the enzymes or pathways that make NPs and there was an increasing interest in the way that organisms that made NPs varied their concentration after those organisms were challenged by other organisms (see Chapter 8). Scientists still struggling to find a convincing reason why organisms made NPs were particularly impressed by two pieces of general information from the great pile of NPs research data:

- Some NPs had very powerful, specific types of effects on some organisms.
- Some organisms increased the rate of synthesis when they were attacked by other organisms.

Surely, this could not be chance? A paper by Fraenkel was particularly influential among plant biologists. Fraenkel marshalled the arguments that NPs were part of a chemical defence system that enabled plants to defend themselves against herbivores (plant eaters). It was an elegant and timely argument, advanced to a receptive audience. Around this period, plant pathologists were trying to discover more about the way in which plants defended themselves against fungal pathogens. For decades, synthetic chemical fungicides had been known to be very effective at protecting plants from fungal attack and the possibility that plants contained their own antifungal substances was being investigated (see Chapter 8). Horticulturalists and plant breeders had known for many years that some cultivars of a species would resist fungal attack while other cultivars were highly susceptible to infection. It was also known that closely related fungal pathogen isolates could vary greatly in their ability to infect their host species. Maybe highly pathogenic strains of a fungus arose from individuals that had evolved a resistance to the fungicide produced by the plant? Very shortly, after highly toxic chemicals were introduced as control agents in agriculture, evidence that organisms could develop resistance to toxic compounds had begun to accumulate.
Why Do Organisms Make NPs?

The evolution of resistance to a natural control agent in a pathogenic organism and the evolution of new chemical defence chemical in the now susceptible host could be viewed as an ‘arms race’ (a term all to familiar to most adults at the time when these ideas were blossoming). Fraenkel’s ideas were taken a stage further in a classical paper by Paul Ehrlich and Peter Raven in which it was argued that co-evolution between specialist insect herbivores and their host plants could explain why so many insect herbivores were such specialists. An insect herbivore species that had adapted to cope with the specific chemical defences of a particular plant species would have more exclusive use of that resource but this would impose a new selection pressure on the plant species. Consequently, a mutant in the population of that plant species which made a more effective mixture of NPs would suffer less herbivory; it would thrive and be more successful at passing its genes on to the next generation. However, this change in NP composition of the plant would impose a new selection pressure on its insect herbivore species and mutants in those insect species that were better adapted to the new mixture would be expected to appear and subsequently thrive. These cycles of adaptation between the insect and the plant are an example of co-evolution, a process that results in a closer and closer association of one species with another. The beauty of this model was that it seemed to explain why plants produced so many NPs. There were so many different interactions taking place between plants and organisms that attacked them, or were attracted to them, that it was predictable that the many different selection pressures unique to each interaction would result in a very large NP diversification. Furthermore, this co-evolution model would explain why different species of plant produced quite different mixtures of NPs—every interaction would be unique and would have produced outcomes over evolutionary time that were unique to that interaction. This Chemical Co-evolution Model quickly became the accepted paradigm among plant biologist, entomologists and plant pathologists. However, many researchers working on NPs in microbes were still unimpressed by this model. Partly, this was because much less was known about the interaction between microbes, especially in the natural environment. The idea of chemical warfare between microbes certainly seemed superficially attractive in explaining why penicillin had been discovered but microbiologist had already learned that finding powerful antibiotics was not as easy as the chemical arms race would suggest (see Chapter 7). Many microbiologists worked for companies that had invested large amounts of money seeking antibiotics and, unlike NPs researchers working on plant-plant interactions or insect-plant interactions, they had purposefully surveyed the occurrence of antibiotics in a huge range of microbes and they had found very few. However, gradually some of those working on microbes began
to rally around the Chemical Co-evolution Model, even if many remained sceptical. Commenting on this continuing scepticism Demain\textsuperscript{11} said:

It has always amazed me that the importance of chemical compounds in ecological interactions between plant versus herbivore, insect versus insect, and plant versus plant has been universally accepted, but the importance of antimicrobials in microbial interactions has been almost universally denied.
(p.101) Constitutive versus inducible chemicals

In evolutionary arguments, it is always useful to try to identify where ‘costs’ arise and where ‘benefits’ accrue. For those not familiar with biological systems, it may be easier to think about the concepts using simple manufacturing economics as an analogy. A producer of any product has a number of costs—the cost of materials needed to make the product, the cost of the machinery needed to make the product, the cost of the energy needed to operate that machinery, the cost of any control systems (machines or people) that optimise the production process, the cost of maintaining all parts of the factory, the cost of keeping any stock and so forth. The benefits in such cases are easier to list—the resource that the company obtains from the market and from its customers. In the perfect market place, if two companies make identical products, the company with the lowest costs will thrive and eventually drive its competitor out of business.

If an organism is making any chemical or structure to serve a particular purpose, it clearly pays to make it only when needed (see also Chapter 8). If one makes a defensive chemical continually, there is an ongoing cost but the benefits may only occur irregularly. A mutant that only makes the chemical when the need for defence is sensed will clearly have increased fitness—equal benefit but lower cost. This concept was very influential in the case of the chemical defence of plants to fungal attack. Indeed, the definition of phytoalexin was ‘an antifungal chemical made by a plant in response to fungal attack’. The idea gained wider acceptance somewhat later in plant–insect interactions but it is now generally regarded as an important feature in judging whether a particular chemical is involved in a particular interaction. The fact that an organism makes a particular chemical in response to a specific challenge has been taken to imply a powerful connection that indicates purpose (as we shall see later in Chapters 8 this logic is maybe not as good as it seems). The concept of the inducibility of NPs so nicely complemented the Chemical Co-evolution Model that both were strengthened by the mutual support.
The problem at the heart of the Chemical Co-evolution Model
As evidenced by the quotation at the beginning of this chapter,\(^\text{12}\) by the end of the twentieth century, there was still no winner in the race to provide a universal model to explain the evolution of NP chemical diversity but the Chemical Co-evolution Model was well ahead. Indeed, many of its advocates considered the race won. Certainly, this model had many attractions but it had some worrying weaknesses.

The first weakness was that the Chemical Co-evolution Model was least convincing when applied to the most diverse, most ancient NP producers—the microbes. As explained above, many microbiologists simply did not find the model convincing when applied to microbes because very little real evidence for the model came from studies of microbes. The main evidence arising from studies of microbes was that some (a very, very few) microbial species could make antibiotics, but some microbiologists were much more impressed by the fact that the majority of microbes did not seem to make antibiotics.

A second major weakness for the Chemical Co-evolution Model was that it demanded that every NP has (or had in the evolutionary past) a specific role—every NP should possess an identifiable type of biological activity. Why should this be true? Because in simple evolutionary terms, mutants of an NP-producing organism that synthesised a new NP that possessed beneficial biologically active would be favoured, while a mutant that produced a new NP that was biologically inactive would not be fitter, but because of the extra production costs would be less fit; consequently, that mutant would be lost from the population by selection. This simplified version of the evolutionary theory to explain NP production would predict that any one NP-producing organism should produce very few, highly biologically active NPs.\(^\text{13}\) Yet when one surveys the literature concerning the incidence of biological activity in collections of NPs, the percentage that have potent, specific biological activity is very, very low (usually <1%). For example, after the discovery of penicillin, huge, well-resourced searches were conducted by many groups hoping to find yet more antibiotics. A 10-year study of 400,000 different microbial cultures only found three utilisable antibiotics. Another one-year study of 21,830 isolates found two possible substances with potential as antibiotics.\(^\text{14}\) Once again one can speculate that there might be many more antibiotics being made by
these cultures that were simply missed in the screening methodology but no model should confidently rely on speculation. So, the central problem for the Chemical Co-evolution Model is that it predicts that a very high proportion of NPs will have very potent, specific biological effects, yet the experimental evidence does not support this prediction.

Building a new model to explain NP diversity—the Screening Hypothesis

Instead of trying to sustain the Chemical Co-evolution Model by disregarding evidence which shows that any one chemical structure has a very low probability of possessing a specific, potent type of biological activity, what happens if one tries to build a model of NP diversity which accepts this fact? The result is the Screening Hypothesis, which takes the Chemical Co-evolution Model as its starting point but rebuilds it on proper physicochemical principles. However, a sizeable digression is needed first to convince the reader that potent, specific biological activity is indeed a rare property for any one chemical.
What is biological activity?

The widely used term *biological activity* is so vague that it is virtually meaningless.\footnote{16} Even though the term *biological activity* is one that is widely used, and understood by most biologists or biochemists, it has no precise meaning without a reference point. As (p.103) the great mediaeval scientist and mystic Paracelsus (1493–1541) noted, one cannot make statements about a form of biological activity without taking into account the concentration of the chemical being used when studying the effect. Paracelsus studied the effect of poisons on organisms. He astutely observed that many seemingly innocuous chemicals had adverse effects on organisms when given in very high doses. More importantly, he observed that known poisons had little effect on organisms if given in very small amounts. These thoughts were summed up in the phrase ‘the dose maketh the poison’. Hence, when judging the *biological activity* of any chemical, a reference point is needed as to what concentration should be used in making a judgement. Furthermore, because each species of organism differs from each other, a particular chemical might show some *biological activity* against one organism at a specified concentration but have no effect on many others. It follows from these considerations that it is predictable that if one tests a particular chemical at a very high concentration in a very wide range of organisms, there will be a high probability that that chemical will be found to have some effect on some organism under some conditions. However, as one reduces the dose of each chemicals being assessed, the fraction of chemicals that produce an observable effect will reduce. Similarly, if one reduces the range of organisms being used to assess the biological activity, the proportion of chemicals showing an observable response will fall. Finally, as Paracelsus would have predicted, if one assessed the effect of low doses of some chemicals on a quiet specific effect on only one species, the chances of finding that any one chemical showing biological activity would be very low indeed. So, the chance of any chemical possessing ‘biological activity’ depends entirely on the way in which one is measuring biological activity. Without specifying the dose being used and the breadth of the organisms being challenged in an assessment, biological activity can mean very different things to different people.
Why Do Organisms Make NPs?

It was this flexibility in the definition of the term central to the argument about the role of NPs that covered the most glaring deficiency of the model—the great majority of NPs have never been shown to possess any form of biological activity against even one organism. Was this because, as advocates of the model argued, the real targets of the majority of NPs had never been identified? For example, a scientist studying the importance of members of a particular class on NPs as insect defence chemicals would not be concerned if only one particular member of this family of NPs seemed to possess potent insecticidal activity against one insect species because that researcher could assume that other researchers would find roles for all the other members of this class of NPs, either defending the plant against another species of insect, against one of the many species of fungi, against bacteria, against nematodes or indeed against other species of plant. Once again the fragmentation of the study of NPs encouraged people to be highly selective with the data being gathered. There simply was no collective responsibility to assemble a coherent model to explain the NP diversity in all organisms.

Remarkably, the clear prediction that all NPs should show unambiguous evidence of some form of biological activity, at concentrations that the producing organisms could realistically achieve, was testable. Indeed, the prediction was being tested daily for the past three decades of the twentieth century by many large pharmaceutical companies around the world as they sought new drugs or by agrochemical companies searching for new insecticides, herbicides and fungicides. Although for commercial reasons, most of these data were not widely available (see Chapters 4 and 7), sufficient data were published to allow some judgement to be made about the probability of any one chemical possessing biological activity. For example, it has been estimated that in excess of 100,000 different, related chemicals (based on the structures of some powerful insecticides—organophosphates) were synthesised by agrochemical companies seeking new insecticides but less than 100 of those chemicals were of commercial value. One agrichemical company (ICI) reported that the chance of finding a single useful product was 1 in 1800 in the crude tests (on whole organisms) being conducted in 1956 and fell to 1 in 15,000 by 1978 when more specific forms of activity were sought. The large-scale screening for biological activity by pharmaceutical and agrochemical
companies provided overwhelming evidence that the probability of finding one strikingly potent chemical was very low indeed—often <0.01%. Because this evidence came from the screening of synthetic chemicals, some questioned its relevance to NPs (read Chapter 4 to explore this assertion more fully). However, the large-scale screening of collections of NPs, although conducted much less often, provided very similar but low hit rates. For example, one screen of the NPs made by 10,000 different microorganisms found only one clinically effective agent. If every plant was making a natural insecticide, why had so few potent natural insecticides been found? If every plant relies on making an NP with fungicidal properties for its defence, why have so few fungicidal NPs been found? So, all large-scale screening trials, whether conducted using synthetic or naturally made chemicals, have shown that the probability of finding a chemical that can specifically target one biological process is extremely low. If we now consider the molecular processes that allow chemicals to bring about their biological effect, this low hit rate can be explained.
The interaction of molecules determines the interaction of organisms—the concept of biomolecular activity.

All large processes are governed by the properties of the lowest level interactions occurring in that process. For example, the greenhouse effect (the trapping of solar energy by the earth’s atmosphere) is a property of the earth’s atmosphere but the characteristics of the atmosphere are the result of the properties of the individual molecules that make up the gases in the atmosphere. Likewise, the properties of those molecules are dependent on the properties of the elements that make the molecules. And so on until one reaches the most fundamental particle that makes up each atom. So, the properties evident at a global scale are highly dependent on properties at lower levels of organisation—the properties are inherited as one goes from the fundamental particle to the largest scale being analysed.

This same concept of inherited properties applies to biological systems. At each level of biological organisation—atom, molecule, organelle, cell, tissue, organ, organism, population and ecosystem—constraints will have been imposed on the higher levels of organisation by each of the properties of all levels below. Because evolution works by selection on options that are available, chemical and physical constraints will be significant constraints on those options. When considering the chemical interactions between organisms, the constraints imposed by the way in which chemicals interact with each other at a molecular level will have been a fundamental constraint on what is seen at the organism level. The interaction between two highly evolved organisms will be no different from the interaction between two very simple organisms in terms of what happens at the level of the interaction of chemical A with chemical B. The more highly evolved organism might process the information it gains by detecting chemical A interacting with chemical B differently from the simple organism, but the basic constraints imposed by the way in which A can interact with B remain. This is why it is important to build any theory about the evolution of NPs on what is known about the way in which molecules interact, because the constraints imposed by those interactions will have been inherited over the billions of years during which NPs have evolved. Whatever effects might be measured in higher organisms that evolved billions of years later, those
effects will still be governed by the same ancient molecular rules. This is why the term ‘biomolecular activity’ was introduced to help understand how NPs evolved:18

The biomolecular activity of a substance is the ability of that substance, when present at a low concentration, to significantly influence the function of a specific protein.

The advantage of thinking in terms of the biomolecular activity of substances rather than their ‘biological activity’ is that it is more discriminating. For example, there are hundreds of substances that show ‘biological activity’ as insecticides but not all chemicals classed as insecticides kill the insects in the same way (there are many ways to kill an organism). One can be more specific by classifying insecticides on the basis of their mode of action. For example, a small subset of insecticides kills insects by interfering with the insect’s acetylcholine esterase enzyme and so the vague term ‘biological activity’ (insecticide) can usefully be replaced by the categorisation based on a specific biomolecular activity (acetylcholine esterase inhibitor). It follows that one form of generalised ‘biological activity’ may encompass one or more forms of biomolecular activity.

What do we know about the basis of biomolecular activity? The study of dyes might seem an unlikely way of beginning a process of probing the way in which chemicals cause their biological effects. However, pioneering studies of the way in which different dyes stained cells (see Figure 6.2 and Chapter 7) provided some important clues about the fundamental processes. Paul Ehrlich (this Paul Ehrlich was a German medical researcher, who lived between 1854 and 1915, and should not (p.106) be confused with the other Paul R Ehrlich of the Chemical Co-evolution Model) and others showed that there was usually a great specificity of the interaction between any one dye and some parts of the cells which they stained. This specificity was associated with the chemical structure—for example, some red dyes might stain one structure in one type of cell, but other red dyes would not do so. This tells us that there is specificity in any interaction between chemicals. It was also noted that the degree of staining depended on the concentration of the dye used—pale colours were produced by low concentrations and denser one by higher doses. It was also noted that the strength of the association between a dye and the specific component of the cell to which the dye ‘bound’ varied; for example, some dyes were easily washed out of a stained cell once the dying solution was replaced with a solution free of dye but other dyes were harder to remove by
washing. Such observations tell us that the interactions between some dyes and their targets are reversible. It also tells us that the rate of reversibility is not fixed but a variable. These principles became clearer when new types of biological activity were explored in the early part of the twentieth century. Ehrlich used the concepts of specificity to guide him in seeking selective toxins. By investigating the effects of hundreds of individual chemicals on the viability of bacteria and higher organisms, it became clear that many chemicals were not sufficiently toxic to the bacteria to give them any potential as agents to kill bacteria but a few chemicals did have potent antibacterial properties and an even smaller proportion of chemicals inhibited the growth of bacteria but were tolerated by higher organisms. So, the numerous studies carried out on the way in which chemicals interact with substances in cells, starting with dyes, then continuing with pharmaceutical drugs, insecticides, fungicides, herbicides and endogenous hormones have produced some basic features that can be said to characterise the way in which a small molecule interacts with a large molecule to produce a biological response.

*Interactions are highly specific.* Of the thousands of molecules that enter your nose as you walk around a garden, only one, would smell of peppermint. When your doctor gives you penicillin, it will not harm your cells and it will not even kill all the bacteria in your body but it will kill those responsible for your treatable infection. If you want to kill the broadleaved weeds in your lawn, your garden centre will sell you a product that will target those weeds but which will not kill the grasses that make up your lawn. These examples of specificity underpin the pharmaceutical, agrochemical and veterinary drug industries and to some extent the food and fragrance industries.

*There is a known relationship between the dose of a chemical and the magnitude of the response generated.* When studying the effect of a chemical on any biological system, there is a characteristic relationship between the amount of a substance administered and magnitude of the response induced. This relationship is called the dose–response curve (Figure 5.1).
Most biological effects are reversible in the short term. The majority of effects of chemicals on biological systems are to some extent reversible in short-term experiments. For (p. 107)

Figure 5.1. An ideal dose–response curve. Note the horizontal x-axis is plotted on a logarithmic scale while the y-axis is plotted on a linear scale. Consequently to double the response from 20% to 40% requires very much greater than a doubling of the dose applied. A consequence of this is that when gaining such data experimentally, it is much easier to gain statistically sound data where the curve is steepest (20–80% maximum response) than at the extreme points of the curve where large changes in the dose cause little change in the response. When doing an experiment on whole organisms to produce such a dose–response curve, this ideal relationship is sometimes not found due to the fact that the applied substance might have difficulty gaining access to the site of action, metabolism (the rate of which will have a different dose–response relationship) might be influencing the result and the ability of the organism to show a 100% response might be limited.
example, if one administers a sublethal dose of a chemical that inhibits the rate of multiplication of a bacteria growing on an agar plate, the effect will be lost if the chemical is washed from the plate—the bacteria will start to multiply again once they are no longer experiencing a significant dose. An example that most people will be familiar with is a local anaesthetic—its effect wears off after some time as the concentration of the chemical at the place where it acts decreases. The majority of pharmaceutical drugs and agrochemicals act reversibly.

These factors are each providing clues about the way in which small molecules (roughly speaking chemicals with a molecular weight of less than 1000) cause their biological effects if indeed they show any effect. One well-established chemical law, The Law of Mass Action, underlies these findings and, as we shall see a little later, this law guides us to the new model of NP diversity.

(p.108) The Law of Mass Action, binding sites and receptors—understanding why specific, potent biological activity is a rare property for any one chemical to possess Binding sites. Why is specific, potent biological activity a rare property? An important clue came from biochemists studying how organisms transformed chemicals using enzymes. Enzymes had been shown in the nineteenth century to be proteins. In the early decades of the twentieth century, the interaction of the enzyme with its substrate (the chemical on which the enzyme acted) suggested that every enzyme must have a unique structure that allows it to ‘bind’ very tightly to its substrate. Part of the enzyme 3-D structure has been selected by evolution to complement the 3-D structure of the substrate. The better the complementation between the enzyme and substrate the tighter the binding (in other words, the enzyme will bind a substrate at even lower substrate concentrations). When biochemists studied the ability of enzymes to accept different, structurally related substrates, they usually found that most enzymes involved in the basic metabolism (see Chapter 9) were very substrate specific—the enzymes only accepted a very limited range of substrates. Furthermore, some chemicals very closely related to a substrate would bind to the enzyme quite strongly but the enzyme could not transform the chemical into a new structure and such a chemical, if added together with the normal substrate, would inhibit the enzyme action on its normal substrate. Such inhibitory substrate analogues sometimes found a use as competitive inhibitors to reduce the capacity of that enzyme experimentally. These interactions between the binding sites on enzymes and the small molecules that
associate with those binding sites became the models for the way in which any small molecule associated with a protein in a specific manner.

In the 1960s, physiologists became interested in how hormones could bring about their effects in organisms, even though the concentrations of the hormones in the cell were often extremely low (micromolar or nanomolar). Because of the importance of hormones in human physiology, and because people with abnormal hormone physiology show serious health impairments (diabetes, thyroid deficiencies, etc.), close attention was paid to the way in which cells sensed hormones. Many chemicals had been made which were structurally similar to known hormones and their biomolecular activity (their ability to mimic the effect of the hormones) determined. It was clear from such studies that every type of hormone-sensitive cell had very great powers of discrimination; the cells would sense only a very limited number of hormones or their synthetic mimics. It was also known that the interaction between these cells and any added hormone was a reversible one—the effect of the added hormone could be reduced or abolished if the cell was exposed to a hormone-free solution. Because all these features were shared with the well-known enzyme–substrate interactions, it was postulated that hormone-sensitive cells must contain specific proteins (hormone receptors) which could associate with particular hormones. Because the hormone was unchanged by the association with the protein, the hormone was not described as a substrate but it was termed a ligand.

(p.109) The discovery and full characterisation of several different hormone receptors led to a greater appreciation of the way in which cells could detect and respond to any chemical. This knowledge, taken together with the understanding of the way in which enzymes associated with substrates, made it clear that the association of small molecules with proteins followed a basic set of fundamental principles, principles that could usefully guide studies of the way in which drugs act on cells, the way in which herbicides influence plant growth and development, the way in which fungicides act on fungal cells and the way in which insecticides act in the cells of insects. In all these cases, the fundamental features of the effect of the biologically active chemical were reversibility, a very restricted range of structures showing high activity and an ability to bring about
an effect even when the chemical was applied at very low doses. All these features were also characteristics of the way in which most NPs are known to act. Hence, it is reasonable to assume that the huge amount of information about ligand–protein interactions can inform our quest to understand the way in which NPs act on cells.

Quantitative relationships in ligand-protein interactions—the Law of Mass Action

The Norwegian chemists Cato Maximilian Guldberg and Peter Waage, between 1864 and 1879, proposed a means of quantifying the rate of a reversible chemical reaction between two substances. Given the reaction is reversible, at equilibrium, the rate at which chemical A reacts with chemical B to give AB is equalled by the rate at which AB decays to give A and B (Figure 5.2). These ideas where developed further by biochemists who recognised that the same principles applied to the reversible interaction of an enzyme and a substrate so that such interactions could be described mathematically in a similar way. The concepts were next applied to the interactions between drugs and the proteins and then to the interaction between hormones and their 'receptors'. The relationship first proposed by Guldberg and Waage, and developed by others, is the Law of Mass Action.

For the sake of illustrating our discussion, let's use the example of a hormone (H) interacting with its receptor (R) (hormones can be considered to be closely related to NPs, indeed some might be considered such as discussed in Chapter 9). Suppose we have several tubes each with the same volume of solution containing the same concentration of a hormone receptor protein and let us assume that we have a simple technique to measure whether each receptor is binding to a hormone at any one moment. Because the interaction of the hormone with the receptor is reversible, and follows the Laws of Mass Action, if one adds a different amount of H to each tube one can measure at what concentration of H there is no significant occupation of the receptors, at what concentration of H is half filling them and which concentration of H just fills all the receptors. Plotting these data on to a graph would provide the equivalent of a dose–response curve (Figure 5.3). This in vitro relationship could provide good clues as to the expected in vivo relationship in some cases. For example, if a cell containing the receptor could be
shown to produce a hormone-specific response and if the hormone could freely enter and leave the cell and was not rapidly metabolised, one would expect the relationship between the concentration of \( H \) in the cell and the magnitude of the hormone response of the cell to follow the same curve measured using the purified receptor.

So, the Law of Mass Action is at the heart of all reversible interactions between small molecules (natural and synthetic) and proteins with which they interact to produce any type of biological response.

**Figure 5.2.** The Law of Mass Action is a simple mathematical relationship between the concentration of the protein which binds the substance, the concentration of the substance and the concentration of the protein currently binding the substance.

\[
\text{Chemical} + \text{Protein} \xrightarrow{\text{on}} \frac{K_{\text{on}}}{K_{\text{off}}} \text{Chemical–Protein}
\]

\[
[\text{Chemical}] \times [\text{Protein}] \times K_{\text{on}} = [\text{Chemical–Protein}] K_{\text{off}}
\]

\[
[\text{Chemical}] \times [\text{Protein}] = \frac{K_{\text{off}}}{K_{\text{on}}} = K_d
\]

**Figure 5.3.** A binding curve is simply a dose–response curve determined at the biomolecular level. In contrast to the dose–response curve determined on a whole organism, it is easier to ensure that the substance has good access to the protein, metabolism can be eliminated and the full range of a response will be available. In this graph, the binding curves of two different substances capable of binding to the same protein are shown. Substance A occupies 50% of the binding sites at one-tenth the
We can carry these thoughts a little further forward by thinking about what would happen if the *in vitro* experiment was used to compare efficacy of several different hormone analogues. Such studies usually find that each analogue has a unique relationship with the receptor. The shape of the curves usually does not differ but the position of the curve on the horizontal axis is characteristic of each analogue. The magnitude of the shift is directly related to the ability of the analogue to bind effectively to the receptor—analogues that fit the receptor poorly need a much higher concentration of free analogue in solution to half fill the receptor than analogues that better fit to the receptor (Figure 5.3). The final piece of useful information that these relationships tells us is that a binding curve usually goes from zero receptor occupied to full occupancy over about two orders of magnitude of the concentration of the active substance. For example, if one found that chemical X started to interact with protein Y when the [X] was 1 micromolar, one could predict that at 100 micromolar X, nearly all the proteins in the solution would at any one moment be interacting with an X molecule and any further increase in X would produce no extra effect (the response saturates).
The Law of Mass Action and the specificity of action of NPs

So the Law of Mass Action informs us about the basic rules that underlie biomolecular interactions. So, when studying some form of ‘biological activity’, this law is governing what is happening. Every cell contains thousands of proteins, each playing a specific role, so in theory each protein is vulnerable to interference by a few small molecules binding to some site on the protein’s complicated 3-D structure. But the Law of Mass Action tells us that if the small molecule is present in solution at a very low concentration it will only be able to occupy sufficient sites on one specific protein if it has an exquisite fit with any vulnerable site. Thus, the theory behind the Law of Mass Action predicts that when tested at a low concentration, there is a very, very low probability of any one chemical structure possessing the right 3-D structure to effectively bind to any one type of protein. So, testing a low concentration of an NP for biomolecular activity against a specific protein target predictably will have a very low chance of finding a significant interaction. However, if tests of the NP are made using a cell-based, rather than a molecular-based, assay the chances of finding some form of biomolecular activity increases a little because each cell contains thousands of proteins. The assessment of ‘biological activity’ might be expected to produce a higher incidence of activity if the testing was conducted on a whole organism because an even larger number of potential protein targets would be aggregated in the assay. If the chemical is screened for activity in many species of mammals or insects for example, the chance of finding an effect increases only a little but because every species contains many highly conserved proteins; one is effectively retesting against very similar or identical proteins in each species. If one tests against a wider range of organisms, the chances of finding potent biomolecular activity increases a bit more but again some proteins are highly conserved across very diverse groups (Figure 5.4). (p.112)
The implications of the Law of Mass Action to the evolution of NPs—the Screening Hypothesis

Given there is a very low probability of any compound (whether made by a human or made by any other organism) possessing potent, specific biomolecular activity, how would this fact have influenced the evolution of NPs? One can build a simple model of the evolution of a metabolic pathway where each postulated step in the evolutionary process is assigned a probability and one can run the model to see how the possible evolutionary outcome would depend on the probability assigned to each process.\(^\text{20}\) At its simplest one starts with one new NP (NP') being made by a mutant in a population that happens to possess a new enzyme that can make NP' from an existing substance X in the cell. One then adds another step in the process so that another mutation arises, which can make yet another novel NP'' from NP' (Figure 5.5). One can assign a probability for each new NP

\[\text{Figure 5.4. Theoretical relationship between number of target proteins and chance of finding biological activity at different organisational scales. The main point being made is that most organisms share a very large number of proteins that have been highly conserved, these proteins being very important to the short-term fitness of the organism. Consequently, if an applied chemical at a particular dose has a 1 in 1000 chance of reducing the fitness of one species then testing the same chemical, at the same dose, on 1000 different species of the same taxonomic class will only increase the probability of finding a chemical capable of reducing fitness very slightly. As one extends the range of species being tested, the probability of finding significant activity only creeps up. The figures given in this graph are invented for the purpose of argument.}\]
having a type of biomolecular activity that is beneficial to the producer but one also has to consider whether another type of mutation might bring (p.113)

about a benefit in a mutant by ridding the mutant of some unnecessary costs. In other words, the chances of a new pathway extension depends not only on the probability of any new substance made bringing significant benefits to the producer, but also on the chances of a mutant arising that saves costs by ridding itself of a redundant or useless enzyme (or indeed a whole pathway). Because it is more likely that a mutation will abolish an enzymic activity rather than produce a useful novel form of enzyme, the potential cost savings by pruning biochemical dead wood will be significant. The reason that the loss of function of a protein by mutation is much more likely than the production of a useful novel protein, are that most changes to a protein structure are likely (p.114) to be detrimental because evolution has already optimised the protein’s structure (see Chapter 9). Thus, there are several probabilities to be considered when thinking about the evolution of NP-producing pathways. At each stage of the pathway extension, the probability of the novel NP possessing useful biomolecular activity remains very low, yet the opportunities for cost savings increases at each stage. Furthermore, it could be argued that the probability of gaining useful biomolecular activity decreases with each step because any new NP made will be structurally related to an existing NP, an NP that the target species will already be co-evolving to resist or tolerate. Studies of the development of resistance to synthetic control agents have revealed that many resistance mechanisms cope with chemically related substances. So, how have NP pathways evolved?
The Screening Hypothesis, first outlined by Jones and Firn in 1991, made the radical proposal that, to compensate for the poor odds demanded by the Law of Mass Action, organisms that gain fitness by making NPs must have evolved to generate and retain chemical diversity. The hypothesis was named to emphasise that organisms making NPs face the same challenges as humans trying to find new drugs or agrochemicals using screening trials. Humans designing a screening trial know that the more chemicals they test, the more chances there are of finding a useful chemical. Humans also know that keeping as much chemical diversity as possible to use in another screening trial, often seeking an entirely different form of biomolecular activity also makes sense.

So, how could NP-producing organisms increase their chances of making and retaining chemical diversity? The Screening Hypothesis proposed that by evolving certain metabolic traits, most importantly abandoning the dogma taught in every elementary biochemistry course or textbook, that enzymes are always substrate specific, one could indeed predict that there could be ways that organisms could maximise the production of NPs.
of chemical diversity and increase the chances of retaining even some ‘redundant’ NPs. The retention of some ‘redundant’ chemical diversity is necessary in order to seed the generation of new chemical diversity.
Enzyme specificity

When students learn about enzymes, it is nearly always stated, very dogmatically, that enzymes will act on only one chemical—they have very narrow substrate specificity. Indeed, when introducing the concept of enzyme action at an elementary level, the analogy of the lock and key is often used to illustrate the concept of specificity. In other words, students are told that for every product found in a cell, there will be one enzyme that has made that product and that enzyme will make no other product. This idea was extended to the idea of one gene—one enzyme—one reaction. There is indeed a considerable body of evidence to support the view that many enzymes do have narrow substrate specificity. However, exceptions were known to this ‘rule’. But more importantly, the types of enzymes used as good examples of the ‘lock and key’ concept were drawn from a rather limited range of examples, mainly examples of enzymes that were involved with the basic metabolism found in a wide range of organisms (see Chapter 9 for a more detailed discussion).

Why do so many well-studied enzymes have narrow substrate specificity? Is such specificity inevitable? Firn and Jones have argued that there is no a priori reason to assume that all enzymes must be substrate specific. They have suggested that high substrate specificity, rather than being inherent, will usually be the result of evolutionary selection. When a mutant organism produces a protein which possesses a novel enzyme activity, any benefit that new enzyme might bring to the organism must arise not from the properties of the enzyme but from the properties of the product that the enzyme produces. A novel protein can only be regarded as an enzyme if there is a substrate available for its action. A mutated enzyme that produces no product imposes the cost of its production on the producer but there can be no benefit; hence, the mutant will be lost from the population. Consequently, new enzymes that possess a very broad substrate specificity will have a greater chance of producing a new chemical, hence have some chance (albeit very small) of benefiting the producer. A new enzyme that can only act on one substrate has a much higher chance of being operationally inactive; hence, such mutants would, on average, be lost from the population. However, once a mutant has gained fitness by possessing a new useful chemical, evolution can act to optimise the synthetic processes leading to that chemical. A
subsequent mutation leading to a modified enzyme with a narrower substrate specificity will result in fewer unwanted products and more of the desirable product; hence, the new mutation might under some circumstances be favoured by selection. Thus, enzyme specificity is very much an evolved characteristic and as explained in Chapter 9, the selection forces to bring about this narrowing of substrate specificity may be huge for one type of metabolism (integrated basic metabolism) but very small in another type of metabolism (e.g., NP-producing metabolism).

So why would a broad substrate specificity enhance the generation and retention of chemical diversity?

Consider the pathway shown in Figure 5.6. The upper panel shows the traditional view of the one enzyme/one product pathway. However, suppose that we relax substrate specificity such that each of the three enzymes can act on any substrate, as shown in the lower panel. The result is that the order in which the changes are made to the starting product becomes unimportant and many different structures can be created by the same three enzymes. Broad substrate specificity not only increases the generation of chemical diversity but it does so at low cost. Because making an enzyme is expensive (a large number of amino acids are needed for every enzyme molecule and the machinery used to make the enzyme consumes energy), this is a significant saving and would predictably help compensate on the balance sheet for the ‘waste’ costs of making some chemical diversity that serves no useful purpose. (p.116)
Broad substrate tolerance would also help ensure that some chemical diversity would be retained. In Figure 5.6, if any of the product that requires the action of all three enzymes for its synthesis endows the producer with enhanced fitness, then the other products with no role would be made initially and would only be lost by subsequent selection. Clearly, there is a balance between the advantages of generating chemical diversity, the advantages of retaining chemical diversity to beget future chemical diversity and the disadvantage of retaining costly redundancy.

Evidence to support this proposition that enzymes involved in NP metabolism would possess broad substrate tolerance.

**Figure 5.6.** The production of NPs using ‘matrix pathways’ was predicted by Jones and Firn\(^\text{13}\) because of the opportunity to produce and retain chemical diversity efficiently. In this diagrammatic scheme, three enzymes (e1, e2 and e3) have access to one substrate. The upper panel shows that if each of the enzymes has a strict substrate specificity, a linear pathway producing three new chemicals would be expected. However, if the three enzymes have a broad substrate specificity then the order of conversion can vary and a matrix pathway will result. Now three enzymes will produce 11 novel substances. Furthermore, such matrix pathways are more robust to the loss of any one enzyme activity (see Figure 5.4).
When, in 1991, the prediction was made that enzymes involved in NP biosynthesis would have broad substrate specificity, there was not a large amount of evidence available to support the proposition. There were possibly several reasons for this paucity of evidence.

1. Researchers tend to find what they seek; hence, if the prevailing view among biochemists was that enzymes were always substrate specific, seeking evidence to challenge such dogma would not be a high priority.
2. It is often difficult to isolate and purify many enzymes involved in NP biosynthesis (many organs or parts of organs rich in NPs often contain material which, released from the cell compartments when the tissues are ground up, inhibit enzyme activity). Only later when it became possible to isolate genes coding for NP-producing enzymes, did it became possible to use molecular biological techniques to add the appropriate gene to an organism that was easier to study. By choosing an organism with a simpler NP composition it became possible to identify more easily the minor products that the enzyme under study might be making.
3. Enzymes that make NPs necessarily act on complex molecules. Such chemicals can be very hard for chemists to synthesise; hence, the range of substrates available to the biochemist to assess substrate specificity was often limited.
4. The techniques available to isolate and characterise the minor products made by an enzyme were insufficiently sensitive or specific.

In the decade after the Screening Hypothesis predicted that some enzymes making NPs might have a broad, not narrow, substrate specificity, extensive evidence was indeed found which was consistent with the prediction. The prediction was never made the focus of a major study; hence, the evidence had to be gleaned from results being published by those researching other aspects of NP metabolism. The problem with seeking evidence indirectly in this way is that there is a tendency to find the evidence being sought. However, even allowing for this possible bias, there is now substantial evidence that some enzymes involved in NP synthesis do indeed have a broad substrate tolerance.

**Mutants with changed NP composition**
There are many garden plants that are closely related but have very different, and very characteristic, smells or tastes. The scented leaf geraniums (*Pelargoniums*), for example, can smell of apple, peppermint, cedar, rose and lemon. Most garden centres sell many type of mint (apple, ginger, peppermint, etc.) which also have very different characteristic smells and it was a study of mutants of mint (*Mentha*) that gives one of the nicest, and most complete, examples of the way in which several enzymes in a sequence leading to an NP pathway can readily accept new substrates. Researchers were seeking cultivars (p.118) of spearmint that were resistant to a fungal pathogen that was reducing the commercial production of this plant in the United States. A mutant was found that showed an increased resistance to fungal attack but, unexpectedly, this mutant smelled like peppermint. The very characteristic smell and taste of spearmint is the result of the mixture of monoterpenes (see Chapter 3) made by that plant. So, a comparison was made of the monoterpane composition of the plants that had smelled like spearmint and plants that had smelled like peppermint. This analysis showed that the two types of plant made different, but related, mixtures of monoterpenes. Yet the mutant was known to be the result of a change to one gene; so, how had the change to the activity of one enzyme brought about several changes in chemistry? It was found that the mutated gene was an enzyme that added a hydroxyl group to the 3-position of the cyclohexene ring of limonene while the wild-type hydroxylated the 6-position (Figure 5.7). All the other new products made by the ‘peppermint’ were the result of the fact that all the downstream tailoring enzymes in the peppermint accepted the new 3-hydroxy substrates to give an array of new products. Clearly, the downstream enzymes were able to accept 3-hydroxy substrates or 6-hydroxy substrates. The appearance of an unexpected novel product in the peppermint suggests that a further elaboration of one of the newly created products by some unidentified NP enzyme, from another pathway, generated further chemical diversity. This is a fine example of how a single gene mutation can generate several new products and how new diversity can be propagated in unexpected ways.

**Matrix pathways**
If some enzymes involved in NP biosynthesis can accept more than one chemical as a substrate, the traditional view that metabolic pathways will be linear sequences becomes questionable. The Screening Hypothesis predicted that pathways would be found where the same enzyme might participate in two different linear sequences. The hypothesis also predicted that there might be more than one route to a given product depending on the sequence of enzymic steps—as illustrated conceptually in Figure 5.6. Both of these predictions have been verified in biosynthetic routes to a number of NPs. One such example comes from studies of the pathways leading to flower colours (see Chapter 9 for a discussion as to whether plant pigments are NPs), in particular the *anthocyanoside* flower colours. For example, in petunia flowers, three enzymes (F3H, F3′5′H and F3′H) can produce five different products (eriodictyol, pentahydroxyflavone, dihydromyricetin, dihydroquercetin and dihydrokaempferol) from naringenin. Studies of the flavonoid 3-O-glucosyltransferase (3-GT) in *Perilla* also provide evidence for its role in a metabolic grid. Another example of a metabolic grid is found in the synthesis of lignin, the complex material found in plant cell walls (see Chapter 9 for a discussion as to whether some plant cell wall constituents should be called NPs). A scheme for monolignol biosynthesis has been proposed, where the three enzymes CAOMT (caffeic acid O-methyltransferase), CCoAOMT (caffeoyl-CoA O-methyltransferase) and hydroxycinnamate CoA ligase have sufficient substrate tolerance that they each act on more than one substrate to create a matrix of transformations. The compounds that (p.119)
Why Do Organisms Make NPs?

give the brassica crop products (cabbage, brussel sprouts, broccoli, cauliflower, salad rocket, etc.) their characteristic smells and flavours come from a group of chemicals called the glucosinolates (see Chapter 3). In excess of 100 glucosinolates are known and this diversity of aliphatic glucosinolates is thought to result from a grid of conversions using a limited number of enzymes involved.

Chemical reactions or rearrangements

Figure 5.7. There are many examples now known of the synthesis of NPs via matrix pathways (see also Figure 9.3). However, a nice example of the benefit of such flexibility was revealed when a mutant of spearmint that had smelled more like peppermint was studied.24 A comparison of the terpenes in both plants revealed that the single gene mutation had not resulted in a single chemical change but multiple changes. In the mutant plant, a hydroxyl group was added to the 3-position of the cyclohexene ring of limonene while the wild-type hydroxylated the 6-position. Some of the other wild-type tailoring enzymes in the mutant did not discriminate fully between the 3- and 6-hydroxylated products so a new family of NPs were produced which gave the mutant plant an odour of peppermint.
One of the key differences between chemical methods of making compounds and enzymic syntheses is that the use of chemical reagents tends to create a number of products instead of the usual single product produced by enzymes (see Chapter 4). However, evolution has come up with a way that enzymes can exploit the fact that certain unstable molecules will rearrange to produce multiple products. There are only a few such enzymes currently known to catalyse the synthesis of such unstable molecules but they do generate a wonderfully impressive range of products. A study of the members of the Tpsd gene subfamily in Grand Fir (Abies grandis) found five monoterpene synthases (ag6, ag8, ag9, ag10 and ag11) that were capable of producing multiple products.\(^\text{25}\) The most striking evidence for the ability of enzymes to produce multiple products comes from a study of two sesquiterpene synthases, also in Grand Fir. One enzyme (δ-selinine synthase) produced 34 different compounds from a single substrate and another (γ-humulene synthase) produced 52 products from its precursor. A further interesting example of the generation of multiple monoterpene products comes from a study of monoterpenes in Common Sage (Salvia officinalis), where the search for a (+)-bornyl diphosphate synthase and a (+)-pinene synthase led to the suggestion that both these activities reside in a single enzyme. Similar flexibility is shown by limonene synthase which has been shown to produce multiple products in isotopically sensitive branching experiments and cDNA cloning. In tomato (Lycopersicon esculentum), the sesquiterpene synthase germacrene C synthase also produces multiple products.

Clearly, the different synthetic capacities available within any one natural product pathway will determine the ability of an organism to exploit the opportunity to produce multiple products and the fact that the examples given come from the terpenoid pathway suggest that this strategy could be less universal than the selection of enzymes with broad substrate tolerance. The production of multiple products by an enzyme makes the naming of the enzyme somewhat difficult. Naming the enzyme after the major product produced seems logical but such a convention is arbitrary and even misleading. The product that enhances the fitness of the organism need not be the major product and evolution is most likely selecting the overall properties of the enzyme (and indeed the overall
pathway), not the one product. A convention to denote the ability of an enzyme to produce multiple products would seem to be desirable.

How do the patterns seen in the NP pathways, described in Chapter 3, fit with this model?

Very well indeed, at the time when the Screening Hypothesis was proposed (in 1991), the patterns outlined in Chapter 3 (and summarised conceptually in Figure 3.2) were not clear. Since that time it has been found that, not only do individual NP pathways show many of the metabolic traits predicted by the Screening Hypothesis, but also taken as a group, NP pathways share a common strategy—to enhance the production and retention of chemical diversity at low cost. The predicted features enhance the chances of a single gene mutation in an NP pathway will result in more than one new NP being made.

Criticism of the Screening Hypothesis

Any scientific hypothesis is simply an attempt to provide a theoretical framework that allows people to understand the processes that interest them and also enables (p.121) them to make predictions which can be tested experimentally. However, just because a hypothesis can provide a more complete explanation of observations than previous hypotheses, or makes predictions that are subsequently experimentally verified, does not mean that the hypothesis is valid. Those proposing a hypothesis, and those extolling its virtues, will inevitably focus the attention on the strengths of the hypothesis. Yet it is the weaknesses of a hypothesis that should really be the centre of attention. Critics, who point out the weaknesses of a hypothesis, do a service where they can contribute to the rejection, or the strengthening, of the model. Many critics of the Screening Hypothesis have unknowingly contributed to its development. These critics gave their views at conferences, as anonymous referees of papers or in discussions. Berenbaum and Zangerl were the most focused and forthright critics and their views are discussed below because their criticisms covered all of the themes which preoccupied other critics.

Is biological activity a rare property for a molecule to possess?

Given that the most widely accepted paradigm at the end of the twentieth century to explain the evolution of NPs was based on the assumption (usually unstated) that all NPs were
biologically active, Jones and Firn's questioning of this assumption was inevitably provocative. Berenbaum and Zangerl argued that the vague definition of the term *biological activity* was the fundamental flaw underlying the Screening Hypothesis. By focusing attention on this issue, the critics served their purpose because Firn and Jones were forced to build a more substantial argument based on the concept of biomolecular activity. The huge amounts of data gathered in the 1990s using cell-free screening (see Chapter 7) showed that for any one type of biomolecular activity, many thousands of randomly selected molecules would have to be screened in order to find one which had high, selective, activity. Furthermore, the reasons for the low probability of possessing potent, specific biomolecular activity could be understood in terms of the increasing knowledge of the way in which small molecules associate with proteins. Because it is impractical to assess the biological or biomolecular activity of every NP, sceptics may argue that this fundamental aspect of the Screening Hypothesis remains speculative. However, the argument can be turned round. Is there any evidence that supports the view that all NPs are biologically active?

Maybe the effects of individual chemicals is enhanced in the presence of other chemicals—the magic mixture argument?

NPs never occur in nature as pure compounds. The cells that make NPs are chemically complex. Even if an NP is released as a volatile or into the solution surrounding the maker of the NP, there will be other chemicals present. Berenbaum and Zangerl made another substantial criticism when they suggested that chemicals may be more effective in combination than when administered as a single substance. Given that most screening trials (but very importantly not all) have been conducted using solutions of one chemical only, if mixtures of chemicals were more effective than single chemicals, this would make data from screening trials a very insecure basis for building an evolutionary argument about NPs. The challenge that such thinking presents to the Screening Hypothesis was clearly outlined by Berenbaum and Zangerl. They argued that maybe individual NPs might show little biological activity when given to an organism in pure form but would show much greater biological activity when given in combination with other NPs.
What experimental or theoretical evidence is there which addresses this key issue?

Using the concept of biomolecular activity, the argument can be progressed. If two chemicals show no biological activity alone but do when given in combination there must still be two individual biomolecular activities underlying the effect of each of the chemicals when they are applied in combination. The probability of each compound possessing potent biomolecular activity is not changed by the fact that another chemical is acting elsewhere in the organism. At the biomolecular level, there will be an independence of action except in the predictably very rare case when the two chemicals act on the same protein. Before exploring this further, one needs to consider how two chemicals could have potent biomolecular activity when combined but show little or no activity when assayed alone.

- One simple explanation would be that the effects of the two chemicals are simply additive. Suppose that two very similar drugs have identical modes of action. A patient might find that one tablet of A or one tablet of B was ineffective but one each of A and B had an effect, but so would two tablets of A, two of B. In other words, the combined effect is simply a consequence of the effective dose supplied. Both A and B will behave just like any chemical tested in isolation and the Laws of Mass Action will apply.

- An alternative scenario would be that one chemical X, with high inherent biomolecular activity, is rapidly degraded by the target organism. Owing to this degradation of X, an effective dose cannot be achieved. Suppose, however, that chemical Y inhibits the enzyme that degrades X. If Y is supplied with X, an effective dose of X is achieved. Thus, X or Y might both possess potent biomolecular activity but the activity of each only reveals itself in the presence of the other. Once again X and Y will behave just like any chemical tested in isolation and the Laws of Mass Action will apply to each chemical interacting with the protein with which it interacts.
There are other such scenarios that can be proposed. Evidence exists for both additive and synergistic actions. There is nothing magical about mixtures. The effects of mixtures can be explained in terms of the actions of the individual chemicals, all of which obey the usual physicochemical laws.

So how do these arguments apply to the evolution of NPs? Suppose that one chemical with rather weak biomolecular activity exists in an individual, but that activity will only become evident if another type of biomolecular activity is evolved. The probability of the first chemical possessing any form of biomolecular activity would be low. However, the chance of the combined activity becoming evident is the same as the chance of the second chemical possessing its form of biomolecular activity because one needs that second, very specific form of activity for the activity of the first chemical to be revealed. In other words, the magic mixture argument is an illusion. There is no escape from the fundamental Laws of Mass Action.

There are also reasons beyond the theoretical ones summarised earlier for questioning the idea that it is the mixture of NPs that are more important than any individual roles. Some screening trials have actually been conducted where mixtures of NPs rather than single compounds have been used. The testing of crude extracts of plant or microbial material has often been conducted and such trials usually show that there is a low probability of complex mixtures obtained by extraction showing potent, specific biological activity. Although there is evidence that the probability of finding activity is indeed greater in mixtures, rarely does such activity greatly exceed the predictable additive effects of testing multiple compounds.

Furthermore, it is often overlooked that thousands of screens of complex mixtures have been carried out inadvertently. Many tens of thousands of chemicals have been screened on whole NP containing plants for herbicidal, fungicidal or insecticidal activity. Each plant species will contain its own full complement of NPs. If adding a new chemical to a rich mixture of other chemicals really would increase the chances of finding biological activity, one would expect that such screens for pesticide activity would have a higher probability of success compared to screens using organisms with no NPs. Yet there is no evidence that such screens show higher probabilities of a particular form of biological activity than screens seeking
activity in organisms that are devoid of NPs. Furthermore, it would be predicted that some very specific biomolecular activity would only be revealed when certain chemicals were tested on species of plants rich in a particular NP. The author knows of no evidence to support this prediction.

Furanocoumarins—good models or a group of chemicals with unusual properties?

The furanocoumarins are a group of NPs that are found in the wild parsnip, Pastinaca sativa, and in several other families of plants. In the nineteenth century, it was shown that the exposure of skin to this plant could cause severe skin irritation and blistering. Several scientists, in the early decades of the twentieth century, described similar symptoms but a key finding, by Kuske, was that blistering would only develop if the patient was exposed to light after rubbing the wild parsnip leaf on their skin. The chemicals in the plant that caused this photosensitised effect were identified as belonging to a group that were termed as the furanocoumarins (Figure 5.8).

Because exposure to furanocoumarin-containing plants poses problems to humans and because some of the furanocoumarin family were thoroughly explored as potential drugs, an extensive literature developed. Interesting questions arose concerning the role of such NPs in the plants that made them and the way in which organisms that interacted with the furanocoumarin-containing plants coped with these clearly, powerfully, biologically active chemicals. The co-evolution of the furanocoumarin-containing plants and the insects (p.124)
that could live on the plants was thoroughly studied by Berenbaum and her group. Their data and experience were drawn upon when Berenbaum and Zangerl marshalled their arguments against the Screening Hypothesis. They presented evidence from the furanocoumarins that they argued showed that

- Many members of the furanocoumarin family are biologically active; hence, the probability of a molecule being biologically active was not low.
- Some members of the furanocoumarin family show more than one type of biological activity.

Figure 5.8. Some members of the furanocoumarins family of NPs showing the key feature (red) shared by each structure that allows each to react chemically with some nucleic acid and proteins. Possessing biological activity due to an inherent chemical reactivity is very rare among NPs for reasons explained in the text.
Arguing that the furanocoumarins were a good model for all NPs, Berenbaum and Zangerl proposed that their data presented overwhelming evidence against the Screening Hypothesis. However, they overlooked the fact that the furanocoumarins were not representative of all NPs. Indeed, the furanocoumarins were very unusual among NPs wherein these chemicals were chemically reactive and they bring about their biological actions, not by reversibly binding to proteins, but by chemically reacting with not only proteins but also DNA. Because these chemicals depend on chemical reactivity, any member of the family of furanocoumarins that contains the appropriate reactive groups will possess some ability to react with biological materials hence will show biological activity. Thus, the probability that any member of the furanocoumarins family being biologically active is high because the group is partly defined by the very grouping that is important in endowing the chemical with chemical reactivity. The fact that furanocoumarins possess a broad range of biological activities is predictable because chemical reactivity is less selective than an activity dependent on reversible interactions with specific proteins. Thus, the furanocoumarins are good models for the way in which NPs with high chemical reactivity act but they are unrepresentative of the great majority of NPs which are not highly chemically reactive but act by reversible interactions with specific proteins.

Why are chemically reactive NPs not more common, given the evidence that evolution could have built families of related compounds, each compound having a high probability of possessing a broad range of biological activities? Interestingly, humans have also found it hard to exploit the apparently attractive properties of what were called ‘active site irreversible inhibitors’. It is possible to speculate that there might be high costs associated with the production of a reactive chemical, especially if that chemical has a broad spectrum of biological activity. The organism that must be exposed to the highest concentration of any chemical is the organism that makes the chemical; consequently, making a chemically reactive chemical is a high-risk activity. Any mutant that evolves the ability to make a chemically reactive chemical presumably has a higher chance of being less fit rather than being fitter. Furthermore, even if the producer of such a chemical does not suffer a loss of fitness, the lack of selectivity characteristic of chemicals, such as the furanocoumarins, means that any organism that interacts with the producer may suffer—beneficial insects or fungi would suffer along with deleterious insects or fungi. It would not be surprising if
organisms have not often managed to evolve such that they
gain the advantages of using chemically reactive NPs, yet bear
none of the costs.

What does this chapter tell us about the way science works?
The century old debate about the roles and evolution of NPs
illustrates nicely that the fragmentation of a subject can very
significantly hinder scientific progress. The failure to
construct a satisfactory universal model to explain the
evolution of NP diversity was not due to a lack of effort or
ideas or a lack of equipment or methodologies. One clear
problem was that most of the biochemists working on NP
metabolism had been indoctrinated with dogmas (e.g., ‘one
gene–one enzyme–one product’ or ‘enzymes (p.126) are
highly substrate specific’) that were simply inappropriate for
enzymes making NPs because these dogmas were based on a
type of metabolism that had evolved for a quite different
purpose (see Chapter 9). The fact that the biochemistry
underlying the most economically, socially and historically
important group of chemicals had been relegated to this lowly
position should warn society that scientists can be very
blinkered.

What about the Screening Hypothesis? Exactly where the
hypothesis stands in Schopenhauer's three stages cannot be
fairly judged by one of the authors of the Hypothesis.
However, even if the reader remains sceptical about its
validity, the implications of the model to several other areas of
biology, in the chapters that follow, provide interesting
insights.

All truth passes through three stages.

First, it is ridiculed.

Second, it is violently opposed.

Third, it is regarded as self evident.

—Arthur Schopenhauer

Notes:
(1.) The scientific publishing industry encouraged this
fragmentation because it profited very greatly from it. In the
last quarter of the twentieth century scientific publishing
Why Do Organisms Make NPs?

became extremely profitable, not only for the big commercial publishers (the notorious rogue Robert Maxwell was one of the first to recognise the fact that scientists were easily exploited) but also for the ‘learned societies’. Indeed some learned societies became very wealthy and powerful by exploiting public bodies such as university libraries and government research institutions! Not only did the library subscription rates increase annually at levels well above inflation but publishers looked for ‘gaps in the market’ where they could try to launch ‘must have’ journals that only needed to cater for a small number to become very profitable. By getting an emerging scientific leader to edit a new journal catering to an increasingly topical area, university libraries could be milked a little more. Very healthy profits could be made even with a circulation of only several hundred (profits of $100–200 per subscription were possible on some specialist journals). The author speaks here as one who once took an active part in this trade.


(3.) Try making a list of all the different foods you have eaten in the past 24 hours and think of the challenges that you have given your biochemistry. Hundreds of NPs must have entered your system, most of which will be chemically unknown and of unknown toxicological consequence. Clearly, human experience has eliminated from the diet those foods that overburden the human body with harmful NPs. However, it is worth knowing that some plants (e.g., garlic) contains some very toxic NPs, chemicals that were they made synthetically, would not be allowed to be added to food.

(4.) This problem with the use of the term ‘waste’ is especially topical because there is a sudden enthusiasm by some people to gather up this ‘waste’ to be processed into energy for human use. Humans can burn cow dung or allow the dung beetle to use it. But if humans take it all, predictably the dung beetle population will fall. The same applies to ‘waste straw’ in fields (currently used by soil organisms), ‘waste timber’ in forestry (currently sustaining a range of species in forests) or any other form of ‘waste’. This is obvious yet even some respectable scientists choose to overlook the problem because money is available to divert ‘waste’ into human use.

(6.) Davies J. (1990). What are antibiotics? Archaic functions for modern activities, Molecular Microbiology 4, 1227–32


(8.) Müller had shown in a brilliant, simple experiment that drops of water in contact with infected pea pods were more inhibitory when added to a fungal culture than drops that had been in contact with uninfected pea pods. The antifungal chemical produced by peas was isolated, characterised, and given the name pisatin. The work was highly influential and became a model for many other studies of the phytoalexins (antifungal chemicals that were made by plants in response to fungal attack). See Chapter 8.

(9.) Insects that were resistant to the concentrations of hydrogen cyanide, which was used to treat fruit trees in California to kill overwintering pests appeared within years of the adoption of the technique in the first decade of the twentieth century. The tally of pests and diseases resistant to specific control agents rise annually. About 500 insect pest species have evolved some resistance to some insecticides. Strategies to retard the evolution of resistant fungal strains are now a normal part of any programme to develop a new fungicide simply because a fungicide which fails to provide adequate control on a disease after only a few seasons use is commercially doomed.


Why Do Organisms Make NPs?


(15.) Unfortunately, unknown to the authors of the Screening Hypothesis to explain NP diversity, the term Screening Hypothesis was used by educationalists to postulate that education acted as a filter to screen those that can be trained. That version of the terminology currently wins a Google search.

(16.) The term biological activity is a term that has little more scientific value than a term like big. The adjective big is only useful in scientific debate when it is given a reference point. If an organism is identified as being big, is it big relative to others of the same species, big relative to all species or simply big relative to humans? Big is a useful term if it is given a context and so is the term biological activity. As of late 2008, a Google search for ‘biological activity’ finds over 5.5 million hits so the term may be vague but it is well used.

(17.) There are some natural insecticides known but only the pyrethrums show useful selectivity. The old natural insecticides, such as derris, rotenone and nicotine, are now rarely used commercially because they are considered more environmentally damaging than synthetic alternatives. The author knows of no commercially significant NP fungicides.

(18.) Firn RD, Jones CG. (2003). Natural products—a simple model to explain chemical diversity. *Natural Product Reports*, 20, 382–91
The choice of the word ‘bind’ to describe the association of a small molecule with a larger protein molecule is an unfortunate one because in English the word ‘bind’ can mean a physical link (e.g., to tie together physically with string), but it can also mean simply to unite two entities in some way without any physical link (e.g., ‘people are bound by family ties’). The great majority of interactions between small molecules and proteins do not involve any physical joining, they are a much weaker interaction. There are a few examples of small molecules acting irreversibly by chemically linking to their receptor (see 32) but such interactions are very rare in nature.


(27.) There was a definite reluctance of many working on NPs to admit that studies on the biological activity of synthetic chemicals could provide any useful information about the biological activity of NPs as a group. The fragmentation of the subject again allowed a group to ignore a huge amount of data simply because it did not fit their preconceived ideas. Partly this was because there was a lingering idea that NPs were somehow different from synthetic chemicals (see Chapter 4).

(28.) Possibly, the most extensive screening of NPs ever undertaken was conducted by the US National Cancer Institute, starting in 1960. Over two decades, 114,000 extracts from 35,000 plant samples (from over 12,000 species) were screened but less than 1% showed selective anticancer potential. One assumes each sample must have contained tens or hundreds of NPs so the hit rate was really much lower. However, the bioassays used have a very questionable relationship to any functional significance of endogenous NPs because plants do not form cancers in the manner that animals do (see Chapter 8).

(29.) Suppose when supplied at 1 micromolar, chemical A alone causes a 25% inhibition of a process and chemical B alone causes a 25% inhibition. But if when both chemicals were given at 1 micromolar in combination and the resulting inhibition was 50%, the effects of A and B would be said to be additive but if the inhibition was >50% then the effect would be said to be synergistic.
(30.) The view that the biological testing of chemicals in isolation from other chemicals may underestimate the potential of that chemical to cause harm has been advanced by many worried about the toxicity of synthetic chemicals (see also Chapter 6). For example, some environmentalists have argued that the toxicity testing, conducted as part of the accreditation process needed before any pesticide is sold, is fundamentally flawed because such tests do not take into account the possible interactions that could occur when multiple pesticides are used (and some crops are indeed exposed to several herbicides, fungicides and insecticides during a growing season). Likewise, the evaluation of the safety and efficacy of drugs has been questioned because each drug is studied largely in isolation from other chemicals to which the patient may be exposed. These criticisms have increasingly been taken seriously by regulatory bodies and worries have been expressed in the European Union that while a great deal is known about the toxicity of chemicals when they are used alone to treat organisms, insufficient information is known about the effects of exposure to multiple chemicals. Given that there maybe 80,000 synthetic chemicals released into the environment, the scale of the problem becomes apparent. The EU REACH regulations seek to address this issue with a further, very expensive evaluation of the toxicity of many chemicals currently in use. As discussed in the main text, organisms that make or are exposed to NPs will inevitably have been exposed to very complex mixtures of NPs.


Some non-scientists, and sadly too many scientists, seem to think that science advances only when new techniques or equipment allow novel experiments to be conducted. The media tends to encourage this view because expensive kit always looks impressive (the finest recent example is the Giant Hadron Collider at CERN). However, sometimes it is ideas that are needed, and ideas are hard to capture visually and often difficult to explain in simple terms. Worse still, ideas cost nothing; hence in a society where money is everything, ideas seem unimpressive. This attitude has even infected science management in many countries where schemes have been set up to identify the ‘best’ science by judging the cash inputs rather than the long-term scientific outputs. It may interest some readers to know that the author of this book has never received any financial support for any of his work on NPs.