

Exploiting insects in the search for new drugs

Insects represent a vast, untapped source of chemically diverse and potentially useful compounds.

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It probably isn't the first thought that runs through your head as you swat the latest summer housefly to invade your kitchen, but insects represent the world's biggest success story (Figure 1). At present, the estimated figure for total living species on Earth is ten million, of which a mere 3 per cent are vertebrates and over 60 per cent are insects. Insects are by far the most diverse group of organisms on Earth (Figure 2). Despite this, the scientific community worldwide has yet to give this essential part of Earth's biodiversity the attention it deserves.

So, apart from an uncanny ability to avoid a rolled-up newspaper, what makes insects so successful? The answer lies in the powerful defence systems that they have evolved over millions of years. These rely mainly on the synthesis of peptides and organic small molecules with predetermined pharmacological activities. Each insect species produces a distinctive set of functional molecules as part of their normal physiology. These molecules are used to capture prey or defend against predators, enable survival in inclement environments, protect against adventitious infections, or control cell division and cell-cycle regulation during metamorphosis. Although insects don't possess anything like the same range of different immune cells as humans, they have had 500 million years to finely tune their immune responses. In comparison, our ancestors *Homo sapiens* only arrived around 120,000 years ago.

A fundamental part of immunity

To the outsider, the insect innate immune system may appear second-rate in comparison with its

human counterpart as it is unable to produce either antibodies or antigen-specific lymphocytes like the human adaptive immune response. The two systems, however, are not as different as people might think. Many insect systems have human equivalents, which can be exploited by companies; for example, the fruitfly *Drosophila* can be used for identifying disease-linked genes in the development of human therapeutics. In the case of the innate immune system, templates have also been conserved from primitive life-forms to humans.

The human innate immune system is far from obsolete, but works in tandem with the adaptive system. Its importance in humans can be illustrated by the example of patients with severe burns. Not only is their skin barrier disrupted, but the skin's antimicrobial peptides

Figure 1. Insects are by far the most diverse group of organisms on Earth and represent an untapped source of potential drugs.



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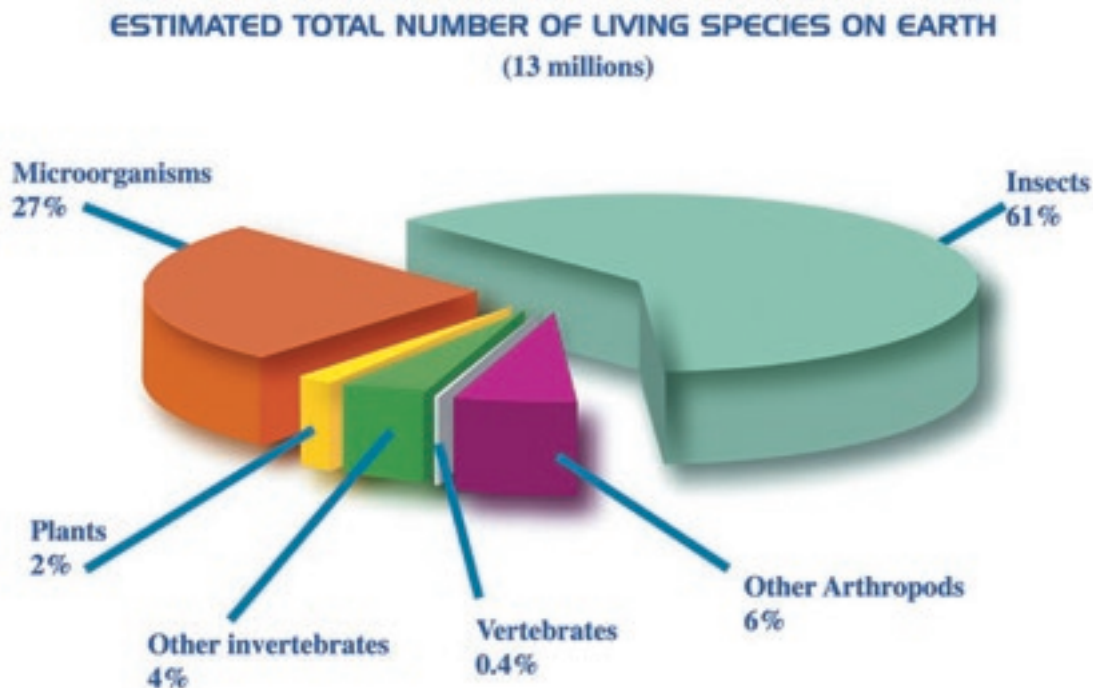


Figure 2. The scale of insect biodiversity.

and cells such as macrophages are also lost, making patients much more vulnerable to infection. Although insects rely on innate immunity alone, the molecules involved have been selected to carry out specific functions by half a billion years of evolution.

Looking to nature

Currently, drug discovery is dominated by High Throughput Screening (HTS) of compounds of small molecular weight. HTS has been developed to process rapidly large numbers of these compounds, but screening assays rely on more and more compounds to feed the discovery engine. Combinatorial chemistry partly answers this need. The technologies underlying combinatorial chemistry, however, limit the chemical space that can be explored using this method of library development. It is increasingly recognised that the key to successful drug discovery is not only the number of compounds screened, but also the structural diversity of the libraries used in the screening process. More diversity is needed to prevent new libraries from becoming merely a variation on an old theme.

In contrast to combinatorial chemistry, natural products provide a wealth of small molecules with drug-like properties and with incredible structural diversity. A comparison of published databases of natural products and synthetic chemicals has revealed that almost half of the chemical skeletons found in natural libraries are not present in libraries of synthetic chemicals. This is despite the fact that natural products have been the source of many of the

best-known pharmaceuticals currently on the market – for example, aspirin, morphine, quinine, digitoxin and ephedrine. Indeed, approximately 85 per cent of the current treatment regimes of 80 per cent of the world's population are based on natural products.

Natural products, in addition, continue to be an important source of new drugs, such as the ACE inhibitors developed from snake venom. An extract from the dry leaves of *Artemisia annua* (sweet wormwood), artemisinin, acts as a blood schizonticide and kills malaria parasites. The immunosuppressants cyclosporin, sirolimus and tacrolimus (the latter two both from bacteria) also come from natural sources, as do the anti-cancer agents paclitaxel, docetaxel and camptothecin. Paclitaxel, for example, was originally an extract of Pacific Yew bark, discovered to have anti-tumour activity in the 1960s during a large-scale screening programme of 35,000 plants sponsored by the US National Cancer Institute (NCI).

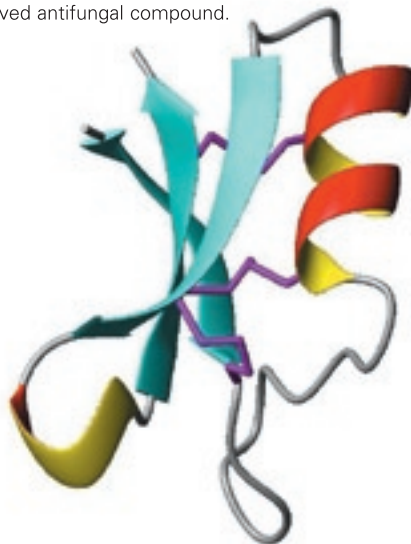
A logical approach to drug discovery

As indicated by some of the examples above, efforts to exploit natural products in drug discovery have largely been dominated by plants, microbes and marine organisms. However, plants and microorganisms represent less than a quarter of all known species on earth, whereas insects and arthropods represent more than two thirds of all species. The added diversity of insects is clear. Whilst plants have generally been employed because they are more readily available, insects offer a huge resource of potentially useful and chemically diverse compounds.

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Figure 3. 3D-structure of ETD-151, Entomed's insect-derived antifungal compound.



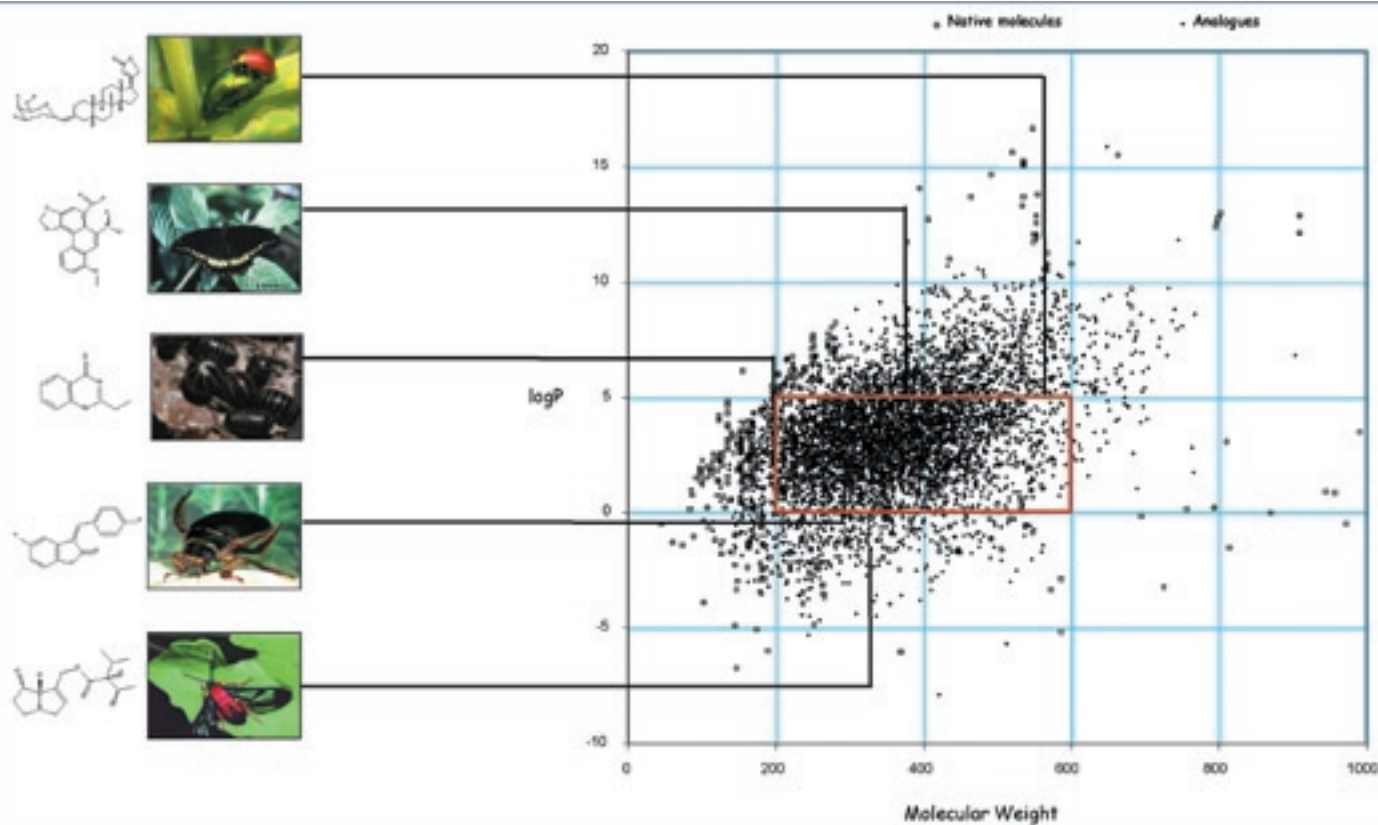
The use of insects can also lead to a drug discovery approach that is far more logical than merely screening a huge library of molecules for ligands to a target. As they have evolved to carry out a specific function, the pharmacological activity of insect molecules is already determined before they are individually identified. Researchers can therefore look for a molecule that has pre-programmed activity, rather than screening a random library of compounds. The risks associated with looking for small molecules against novel targets can also be reduced by looking for novel insect molecules for targets that are already validated in man.

The advantages of evolution

As insect immune molecules have been specifically designed and evolved over millions of years to carry out their functions, they have several advantages over other potential lead compounds. Their high level of specificity, for example, for microbial cell membranes, means that they have low levels of toxicity and are also highly efficient. Current antibiotics work by, for example, inhibiting protein synthesis or nucleic acid synthesis or disrupting the cell wall. To achieve this, they target very specific receptors – enzymes or proteins – which can make them vulnerable to the development of resistant strains. Insect-derived peptides utilise modes of action that should restrict the development of resistant strains, as they have a much broader target – the microbe cell membrane. In addition, they are active against a wide spectrum of microorganisms, with a very rapid bactericidal effect. Insect-derived antimicrobial peptides also demonstrate very low toxicity in mammalian cell lines *in vitro* or during *in vivo* studies. For example, French drug discovery company Entomed's lead compound, ETD-151 (Figure 3), an antifungal derived from a species of moth, has been administered to animal models by 24-hour continuous intravenous infusion at doses up to 600 mg/kg with no signs of toxicity.

Many insect-derived molecules, such as Drosomycin, are also resistant to degradation by proteases. This was the first inducible antifungal peptide to be isolated from insects and, to date, has only been reported in the fruitfly *Drosophila melanogaster* (1). Drosomycin includes extra disulphide bonds, giving the peptide a highly compact shape. This provides the molecule with a remarkable level of resistance to degradation by proteases both *in vitro* (2) and *in vivo* (3).

Figure 4. The Entomothèque™ is a unique collection of insect-derived compounds and analogues.



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From insect molecules to NCEs

If companies are to make full use of insects in drug discovery, they not only need pharmaceutical knowledge, but also considerable entomological expertise and connections. Entomed's solution to this is based on Entoweb™, a network of exclusive collaborations with a number of selected entomology centres. These are situated around the equator, where much of the world's insect diversity is concentrated, giving the company access to a huge variety of novel molecules originating from insects. The company has used these to develop a unique collection of insect-derived compounds and analogues, the Entomothèque™ (Figure 4).

Entomed currently has ongoing projects to identify new organic small molecules. Potential new lead compounds can be discovered via three different means. Firstly, new chemical entities can be obtained directly from insects. Once insects have been obtained via the Entoweb™, they are challenged with a variety of microbes in order to trigger their immune systems. This results in the release of peptides into the haemolymph (the insect equivalent of blood), which can then be separated using High Performance Liquid Chromatography (HPLC) and screened for their efficacy against a selection of seven different microbes as well as other targets. The fractionation and purification process is also adapted to identify small molecules, which are submitted to the same screening battery. The most effective hits can then be structurally characterised and modified using molecular evolution or chemical lead optimisation to improve their activity profile, for example, against specific human pathogens. Secondly, once families of molecules that are unique to insects have been identified, libraries can be synthesised based around these scaffolds. Lastly, commercially available libraries can be scanned for insect-like molecules to identify novel uses for existing compounds.

These processes are yielding a variety of potential drug candidates, from anti-microbial small molecules to anti-cancer molecules and immunomodulators. The potential for new therapies does not stop there, as illustrated by a recent collaboration with Achillon Pharmaceuticals, which is focused on the discovery and development of novel anti-virals from the Entomothèque™.

Conclusions

Although there is the potential to discover huge numbers of New Chemical Entities using techniques such as HTS and combinatorial chemistry, the libraries used need sufficient structural diversity to ensure continued success. Insects, with their unrivalled level of diversity, are one example of a previously untapped source. Entomed is well positioned to exploit this source, using a number of validated technologies to rapidly identify and characterise novel molecules and genes, and to assay their biological activity. This represents a much more logical approach to the development of drugs, yielding molecules for pre-validated targets that have

been specifically designed to perform their functions over millions of years of evolution.



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Steve Dawe joined Entomed as Preclinical Director in January 2002. He has extensive experience of drug development, working at Abbott and BASF Pharma as an International Project Leader with responsibility for managing preclinical and clinical development projects in the metabolic and CNS therapeutic areas. As Senior Toxicologist at Boots Pharmaceuticals, he was responsible for conducting long term toxicity and carcinogenicity studies.

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