Ecological Biochemistry

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Advanced article

Article Contents

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Ecological biochemistry is the study of the role of biochemistry and chemistry in the ecological relationships of different organisms in their abiotic environment. In the middle of the nineteenth century, it was found that insect herbivores could be repelled by chemicals emitted by, or eaten in, a target plant. Development of toxicology, biochemistry, ecology as disciplines and of analytical chemistry techniques allowed the molecular basis to be determined in 1968 when cardiac glycosides (cardenolides) of milkweed were shown to cause young blue jays to vomit up partially eaten Monarch butterflies and to avoid butterflies. Numerous chemical signalling systems were soon found for many host–predator and/or host–benefactor interactions of many species. The advent of genomic techniques in the twenty-first century has allowed the genetic basis of these interactions to be examined in recent research, a trend that will also apply for future research.

Introduction

The discipline of ecological biochemistry was foreshadowed by the discovery in the middle of the nineteenth century that specific insects were protected from their would-be predators by repellents accumulated from their host plants (Rothschild, 1972). A specific example was the defensive use of unidentified toxins of *Asclepias* species by adult butterflies. These toxins had been absorbed by the plant-feeding larval stage rather than by the adult insect (Rothschild, 1972). However, the sciences of chemistry and biochemistry were not then developed enough to identify the toxins.

The British focus of research in this field of endeavour from 1968 to 2000 concerned plant–other organism relationships (usually involving insects (Brower *et al.*, 1968) and animals (Cooper-Driver and Swain, 1976)), whereas fish were then the dominant focus for Russian scientists (Vinogradova, 1968; Hochachka and Mommsen, 1995). Since 2000, genomic techniques have been increasingly used in all areas of research.

The key focus of the modern field is the chemical, biochemical and genetic mechanisms of the biological interaction of different free-living species. This definition excludes interactions that are symbiotic (a long-term association, e.g. the symbiotic bacteria of the human gut) or parasitic (of benefit only to one organism, e.g. trypanosoma in humans) completely within the internal environment of a living host organism. The effects of anthropogenic pesticides are also excluded but not endogenous or natural ones.

Because there have been many differences in research focus, many similar-sounding research fields exist in which the emphasis is on the field represented by the last word: chemical ecology, biochemical ecology, ecological chemistry, ecological biochemistry, etc. There are many areas of overlap in these fields, which makes it difficult to find complete information (**Figure 1**).

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Monarch Butterfly and Milkweed

A 1968 publication reported that cardiac glycosides (cardenolides) of milkweed (*Asclepias* spp.), upon which the larvae of the monarch butterfly (*Danaus plexippus*) fed, could be identified in the adult butterfly. These toxins

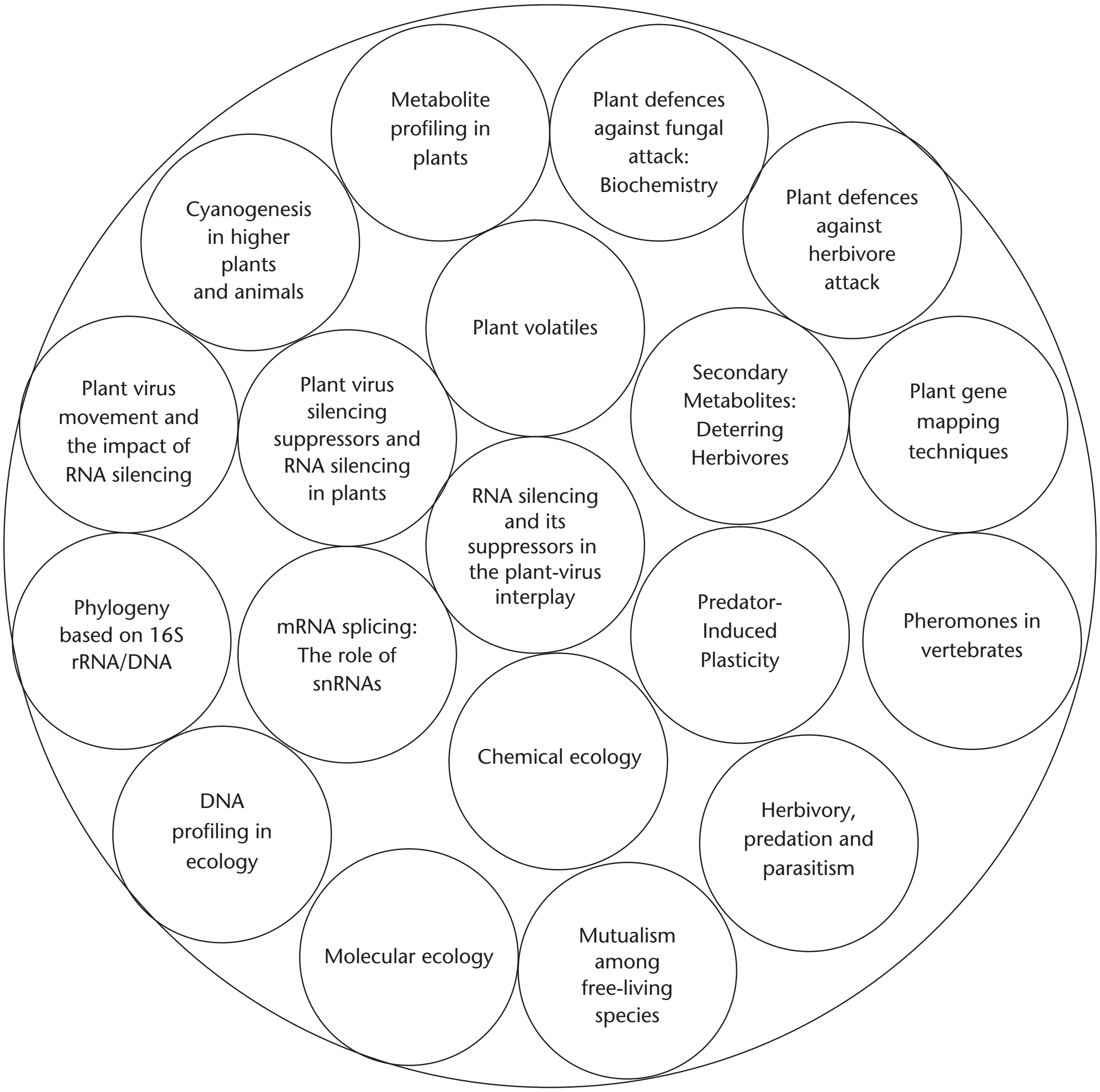

Figure 1 The eLS conceptual circle for ecological biochemistry.

caused young blue jays (*Cyanocitta cristata*) to vomit up partially eaten butterflies and to avoid butterflies of similar appearance (Brower *et al.*, 1968). This set of observations is usually cited as the beginning of ecological biochemistry as a scientific discipline. The adult butterfly has been found to require plants (Boraginaceae spp. and Compositae spp.) to supply the pyrrolizidine alkaloid precursors of its sex pheromones. The flavonol glycosides of *Asclepias curassavica* are also oviposition stimulants (Haribal and Renwick, 1996; Simmonds, 2001). The ability of Monarch proteolytic enzymes of butterfly larvae and caterpillars to promptly digest latex proteins can explain (at least in part) how these insects overcome the defensive proteins and cardenolides found in *Calotropis procera* latex (Rasmann *et al.*, 2009; Pereira *et al.*, 2010). Late instar larvae have also been observed to deactivate the latex defence of milkweeds by chewing a furrow in the leaf midrib or petiole. The larva then feeds beyond the cut where latex outflow is minimal. If a larva does encounter latex exudate during feeding, it often returns to its initial cut to damage the midrib or petiole more extensively before resuming feeding (Helmus and Dussourd, 2005). The biochemical basis of this behaviour is still unknown (Malcolm, 1995; Trigo, 2000; Nishida, 2002; Zhu *et al.*, 2008).

Plants and Insects

Although the monarch butterfly–milkweed interaction was the first documented case of ecological biochemistry, many more cases are now known.

The mechanism involved in the roles of plant fragrances and petal extracts in attracting pollinating bees is a global subject of scrutiny. The neotropical genus *Clusia* L.

(Clusiaceae; Guttiferae was the former name) comprises approximately 300 species that include hemiepiphytes (vines), lianas, shrubs and trees, and while the majority of species are dioecious a few are hermaphrodite or have hermaphrodite flowers. The composition of the volatiles (benzenoids, monoterpenes, oxygen-containing monoterpenes, long-chain hydrocarbons, oxygen-containing long-chain organic compounds, sesquiterpenes, oxygen-carrying sesquiterpenes, carboxylic acids, diterpenes and nitrogen-containing compounds) of fresh petals of 16 *Clusia* species was related primarily to the taxonomic rank of the species, and secondarily to the type of pollinator, there being no dependence on flower gender of the same species (Nogueira *et al*., 2001). Plant sesquiterpene lactones of chicory (*Cichorium intybus*) are feeding deterrants to insects (Rees and Harborne, 1985). Field bioassays demonstrated that the petal extracts were attractive to pollinating bees (*Trigona spinipes*) (Morgan and Wilson, 1999; Kessler *et al*., 2008; Raguso, 2008; Metlen *et al*., 2009; Wink, 2010).

The present research of pathogenesis-related genes and proteins from tree species currently emphasises the resistant genes and the proteins induced during systemic acquired resistance and highlights the ecological and evolutionary significance of defence-related genes from tree species (Veluthakkal and Dasgupta, 2010).

Seeds and Insects

Some seeds contain compounds that repel feeding larvae when a threshold concentration of seed toxin is ingested. Thus, the quinolizidine alkaloids of the Papilionoideae of the legume plant family are toxic at low concentrations to the larvae of the cow pea weevil (*Callosobruchus maculatus*). It has been suggested (Bell, 1981) that canavanine in the more evolved Faboideae (former name, Papilionoideae) substitutes for arginine, with toxic effects to seed beetles such as *C. maculatus* because it differs only by an oxygen that replaces the methylene group in the γ position relative to the carbon that is connected to both the terminal carboxyl and amino groups of arginine. However, a beetle of Costa Rica (*Caryedes brasiliensis*) possesses an arginyl tRNA synthetase that can discriminate against canavanine, and is thus an example of insect adaptation to this chemical strategy (Rosenthal *et al*., 1976).

Some insects carry an odour memory of where they were born. For example, *Megastigmus pinus* seed wasp females from *Abies procera* cones preferred the cone odours of *A. procera* and were significantly repelled by the cone odours of other possible host trees (Luik *et al*., 1999; Roques and Skrzypczyńska, 2003). *M. pinus* females from *Ardea alba* cones preferred *A. alba* cone odour but were not indifferent to *A. procera* cones. *Megastigmus rafni* seed wasps from *Abies grandis* cones preferred only *A. grandis* cone odours. *M. rafni* females from *A. alba* cones preferred *A. alba* cone odour but were also significantly attracted by *A. grandis* cone odours. Neither *M. pinus* nor *M. rafni* responded to the odours of the host-tree needles or to alpha-pinene vapour, but were repelled by limonene vapour.

Worms and Leaves

Plants have developed mechanisms to discourage aerial parts from being eaten by worms. For example, the leaves of *Lathyrus latifolius* contain the nonprotein amino acid L-2,4-diaminobutanoic acid, which at low ingested amounts acts as a phagostimulant but is a deterrent when ingested at high amounts for the larvae of the African leaf worm (*Spodopteris littoralis*) (Bell *et al*., 1996).

Nicotine is a natural alkaloid insecticide and repellent, and yet the larvae of the tobacco hornworm (*Manduca sexta*) feed on the tobacco plant (*Nicotiana tabacum*). The larvae on the plant excreted nicotine rapidly and efficiently (Self *et al*., 1964).

Leaf damage, either by mechanical or by enzymic means, can trigger release of existing defensive chemicals such as alkaloids, terpenoids, phenolics or proteinase inhibitors, or their increased synthesis. New biosynthetic pathways may also be activated. For example, the feeding of the beet army worm (*Spodoptera exigua*) causes the cotton plant (*Gossium hirsutum*) to produce volatile terpenes and indoles (Pare and Tumlinson, 1997; Lucero *et al*., 2009). The trigger chemical is an insect-modified fatty acid of plant origin (Pare and Tumlinson, 1997). The volatile compounds attract parasitoids such as parasitic wasps that prey on the grubs. Tobacco, cotton and maize produce different mixtures specific to the predator. Thus, the specialist wasp *Cardiochiles nigriceps* is recruited to plants infested with its host *Heliocoverpa zea* (De Moraes *et al*., 1998). A similar sequence occurs for the predator *Heliothis virescens*. Plant attraction of carnivores of plant herbivores has been reviewed (Heil, 2008).

The larval codling moth (*Cydia pomonella*) feeding on apple fruit causes induction and release of apple volatiles (esters of C_1–C_6 carboxylic acids, a few aldehydes $> C_5$ and the terpene α-farnesene) but not of any new compounds (Hern and Dorn, 2001). The first infestation always is the most effective induction.

Herbivores and Plants

Clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*) occur in two forms, one that liberates hydrogen cyanide when crushed and another that does not. Slugs and snails prefer the acyanogenic forms of both species. In regions where there are mild winters that allow molluscs to be active (as in the British Isles and the Iberian Peninsula), cyanogenic forms dominate. In Scandinavia and Russia, severe winters keep molluscs from foraging, and acyanogenic forms dominate (Daday, 1954; Jones, 1972). Similarly, the acyanogenic form of bracken (*Pteridium aquilinum*) is grazed by sheep and deer but the cyanogenic form is avoided (Cooper-Driver and Swain, 1976). The biosynthesis of cyanogenic glycosides, glucosinolates and nonprotein amino acids has been reviewed (Selmar, 2010).

The grey kangaroo of southern Australia (*Macropus fuliginosus*) has evolved a tolerance for monofluoroacetate in the legume genera *Gastrolobium* and *Oxylobium* that nonnative animals and humans do not possess. Fluorocitrate is formed instead of citric acid in the tricarboxylic acid (Kreb cycle) pathway, which inhibits aconitate hydratase, leading to respiratory failure in sensitive species. In contrast, the rat kangaroo (*Bettongonia penicillata*) and the bush rat (*Rattus fuscipes*) found only in southwest Australia are tolerant. These data have been interpreted to signify that the grey kangaroo originated in the west, and that the rat kangaroo and the bush rat came from the east (Mead *et al*., 1985).

Induction of defensive proteins in tomato by a natural elicitor, jasmonic acid, caused increases in activities of proteinase inhibitors and of polyphenol oxidase and also in the abundance of four herbivores (caterpillars, flea beetles, aphids and thrips) and insects, in a three-year study (Thaler *et al*., 2001). This research effort is clearly close to a pesticide trial except that it is the plant that has its pest resistance increased by the spraying of a chemical rather than the ‘pest’ being directly affected. Over 90% of previous studies found that herbivore damage to glucosinolate-containing plants led to an increased accumulation of indolic glucosinolates at levels ranging up to 20-fold (Textor and Gershenzon, 2009). The glucosinolate defence system also requires another partner, the enzyme myrosinase, to hydrolyse the parent glucosinolates into biologically active derivatives. The biochemical mechanism of glucosinolate induction involves a jasmonate signalling cascade that leads eventually to increase in the transcript levels of glucosinolate biosynthetic genes. Several recently described transcription factors controlling glucosinolate biosynthesis are activated by herbivory or wounding.

Reviews are available on the chemical defences of poplar (*Populus trichocarpa*; Torr & A. Grey, black cottonwood) to insect herbivores correlated to its genome (Philippe and Bohlmann, 2007), and on chemical information transfer between wounded and unwounded plants (Dicke and Bruin, 2001; Inderjit *et al*., 2005).

Fish

This aspect of ecological biochemistry was the focus of the early Russian research. The fundamental objectives were (1) determination of biochemical adaptations to various abiotic and biotic conditions; (2) determination of the biochemical changes in organisms during metamorphoses accompanied by marked changes in ecological conditions; (3) determination of the biochemical parameters of marine organisms for their ecological classification; (4) elucidation of transfer and transformation of substances and energy through trophic levels within defined ecological systems and (5) determination of the effects of radioactive elements on marine organisms (Vinogradova, 1968). Much of the research effort focused on the lipid profiles of fish, why certain fish have more fat than others, and why some have fats in bound form such as lipoproteins. For example, the fat reserves of *Pontella mediterranea* were found to be 20–25% of those of *Calanus helgolandicus*. Most of the fat reserves of *Limmocalanus grimaldii* were shown to be present as lipoprotein (Vinogradov, 1969). Evolutionary aspects have been of great interest (Sidorov *et al*., 1994). There is a review on the biomarker approach to fisheries (Fromentin *et al*., 2009).

The genetic mechanism of the famous association between the sea anemone *Aiptasia pallida* and its anemone fish *Amphiprioninae* spp. is not known (Cook *et al*., 1988; Morrall *et al*., 2000; Dunn *et al*., 2007b). The biochemical ecology of deep-sea life has also been discussed (Somero, 1992).

There is an abundance of experimental studies and reviews that describe odorant-mediated behaviours of freshwater fish in laboratory microcosms, but research in natural field conditions has received considerably less attention (Johnson and Li, 2010). When findings from laboratory experiments have been further tested in field environments, often different and sometimes contradictory conclusions are found. Examples include studies of sea lamprey (*Petromyzon marinus*) mating pheromones (see also Li *et al*., 2007) and fish alarm substances. Here, environmental context and physiological status influence fish behaviour differently in a laboratory microcosm relative to the field.

Microorganisms and Their Associates

Although the symbiotic bacteria resident in mammalian gastrointestinal tracts may be thought of as examples of ecological biochemistry, these microorganisms are really inside the host and not part of a nonhost environment. Some important cases of the latter include the association of luminescent bacteria with organisms such as squid/octopus and deep-sea fish to act as prey lures, the microorganisms obtaining carbon, nitrogen, sulfur, essential elements and phosphorus sources from the feeding host. These microorganisms only show bright bioluminescence once a critical threshold of microorganism number density is reached. Genes need to be activated for optimum brightness in the host's light organ by a process called quorum sensing that depends in turn on a critical concentration of chaperonins to release *lux* gene inhibitors that need to be uncoiled from specific parts of the bacterial deoxyribonucleic acid (DNA) (Meighen, 1994, 1999; Widder, 2010). The chemical activators for the genes differ according to the type of bioluminescent microorganism.

The interaction between microorganisms of the root rhizosphere and the plant has been reviewed (Arshad and Frankenberger, 1998; Inderjit *et al*., 2005; Hinsinger *et al*., 2011). Although the interactions between pathogenic bacteria and the plant have been most examined in the past with an eye to crop production, research efforts are just beginning on the interactions between nonpathogenic bacteria and the plant (Doornbos *et al*., 2012).

The biochemical basis of how microbial extremophiles exist in hydrothermal vents and frigid Antarctica has been investigated through the characterisation of shock proteins and their genetic controller genes (Nakagawa and Takai, 2008; Ambily Nath and Loka Bharathi, 2011). The role of microbes at great depths >1000 m has been examined, with sequencing of 18S ribosomal ribonucleic acid (rRNA) genes revealing a tremendous diversity of small-sized protists in bathypelagic waters, and high-throughput sequencing of 16S rRNA genes revealing great diversity of bacteria (Nagata *et al*., 2010).

Coprophilous and sclerotial fungi have also been studied as model systems of fungal antagonism and defence by their excretion of active metabolites relative to effects on potential competitors and/or predators (Gloer, 1995). Plant–fungal interactions have also been reviewed (Grayer and Kokubun, 2001; Akimitsu *et al*., 2003).

Global warming has been blamed for the current state of the worldwide bleaching of coral reefs (Hoegh-Guldberg, 1999). Coral bleaching occurs when the thermal tolerance of corals and their photosynthetic ‘symbionts’ (zooxanthellae) is exceeded. Chronic photoinhibition of the zooxanthellae occurs, and the colour centres cannot develop. The biochemistry of the process is just beginning to be investigated (Dunn *et al*., 2007a).

Research on the role of microorganisms of the oceans in global warming has concentrated on their effects on the carbon, nitrogen and sulfur cycles that control partitioning of carbon dioxide in ocean water (Copley, 2002; Beja *et al*., 2002; Melzner *et al*., 2009; Ghosh and Dam, 2009; Schafer *et al*., 2010). Ocean viruses appear to be in control of bacterial blooms in plankton and also of algal blooms like *Emiliania huxleyi*. When ocean algal blooms are killed by viruses or by α-proteobacteria (photosynthetic bacteria that do not produce released oxygen gas, a process called aerobic anoxygenic photosynthesis), large amounts of dimethyl sulfide gas are released from algal dimethylsulfoniopropionate; this stimulates cloud formation, which further cools the clouded area by shielding of light and heat.

The biochemical basis of insect cold hardiness is also being explored as a part of climate change research and to improve pollination and survival of plants under cold conditions especially relative to the first sequenced plant, rice (*Oryza sativa*) and trees (Chen *et al*., 2010; Neale and Cremer, 2011).

Birds

A well known association is that between the rhinoceros bird *Ruphuga erythrorhyncha* and the rhinoceros. The bird pecks off vermin like ticks, dried blood and dead skin, and is fed at the same time. The biochemical and chemical mechanisms in this ecological association are unknown.

Very few ecological biochemical investigations have centred on birds, a clear research need. One such example is the aversion of birds and insects for xylose-high nectars of the Proteaceae but which also attract rodent pollinators (Jackson and Nicolson, 2002).

Intraspecies Communication

A surprising finding is that the Asian elephant and lepidopterans have a common sex pheromone, (*Z*)-7-dodecenyl acetate (Rasmussen and Schulte, 1999; Rasmussen *et al*., 2003; Johnston and del Barco-Trillo, 2009). In elephants it acts as a female-to-male urinary sex pheromone, and lipocalin-like proteins are suspected to be necessary for full activity. Diverse body emissions are also released from temporal gland secretions and the breath of male elephants in musth, and a urinary female-to-male preovulatory pheromone has been identified. Such a result brings together the apparently disparate research areas of sex pheromones in animals.

Economic Uses

How knowledge of biochemical ecology can be used to foster the use of marine natural products as a source of drug discovery has been reviewed (Suffness, 1992; Sennett, 2001; Kennedy and Wightman, 2011). Knowing the basis of attraction and repulsion allows more efficient species collection methods so that large amounts of natural products can be evaluated as drugs or as economic poisons (pesticides). The same aims related to fungi have been reviewed (Eckerman and Graham, 2000; Tahara, 2007a, b). Here, the rationale is that understanding the chemical mechanism of how organisms thrive over others will allow good sources of fungistatic or fungicidal chemicals to be identified through identification and concentration of the secondary bioactive metabolites involved. Examples of such favourable sources include antagonistic fungi, plants with fungal pathogens and mycoparasites. Optimisation of beekeeping is another important economic application of biochemical ecology as discussed earlier (Morgan and Wilson, 1999; Comba *et al*., 2000), as is optimisation of fragrances for humans derived from flowers (Bruyne and Baker, 2008).

The control of pests in crops by chemical ecological principles has been illustrated by utilising a ‘push–pull’ strategy (Pickett *et al*., 1989, 1997; Khan *et al*., 2000; Cook *et al*., 2007). More attractive noncommercial plants are used to attract pests away from the cash crop (‘trap crops’) or to act as associates (‘intercrops’) to repel pests from the cash crop. For example, the two most successful trap crops for minimising stem borer damage of maize and sorghum crops in subSaharan Africa (Khan *et al*., 2000) were Napier grass (*Pennisetum purpureum*) and Sudan grass (*Sorghum sudanensis*). Similarly, the most effective intercrop was molasses grass (*Melinis minutiflora*); two legume species, silverleaf (*Desmodium uncinatum*) and greenleaf (*Desmodium intortum*) were also effective and suppressed the parasitic witchweed *Striga hermonthica* that competed for nutrients and light. Stem borer control was achieved by release of attractant semiochems (signal substance) from the trap plants and repellent semiochems from the intercrops. Another advantage of the trap and intercrop plants is that they also provide forage for cattle, often reared in association with subsistence cereals. In general, such an integrated approach may include protection of the cash crop by host-masking agents, repellents, antifeedants or oviposition deterrants. At the same time, host plant attractants and sex pheromones can be used to stimulate colonisation of pests on trap crops or entry into trap crops that also contain pest pathogens (Pickett *et al*., 1997; Cook *et al*., 2007). Such measures may achieve the desired control of pests through sustainable measures rather than the direct application of chemical pesticides. The intercrop idea has been applied in China for peanut and tea cultivation (Gong *et al*., 2008; Wang *et al*., 2010).

Genetics Research

The genes responsible for encoding proteins involved in interactions external to the organism (including interactions among organisms and between the organism and its abiotic environment) are termed 'environmental response genes' (Berenbaum, 1995, 2002; Hooper *et al*., 2010). Some general characteristics of such genes include high diversity, proliferation by duplication events, rapid rates of evolution and tissue specific or temporal expression. Such genes include those involved in the manufacture, binding, transport and breakdown of semiochems. An example is given by the genes that encode cytochrome P450 monooxygenases involved in the biosynthesis of furanocoumarins (furocoumarins) and toxic allelochems in plants, as well as in their detoxification by lepidopterans (Berenbaum, 2002; Hooper *et al*., 2010).

The genetic basis of the emission of volatile organic compounds by plants has been reviewed (Penuelas and Llusia, 2001; Sardans *et al*., 2011), as has the genetics of hybridisation in plant–herbivore interactions (Orians, 2000; Philippe and Bohlmann, 2007; Textor and Gershenzon, 2009; Hunt *et al*., 2011). The five major genetic levels in the hybridisation of species are parental taxa, hybrid class, ploidy level, chemical class and the genetics of expression (dominance, recessive versus additive inheritance). These factors influence the process of chemical diversification, the potential for introgression, the likelihood that hybrids will facilitate host shifts by herbivores and the conditions that might lead to enhanced hybrid susceptibility and lower fitness (Orians, 2000). Pickett *et al*. (1997) have described the desirability of strategies that employ genetic manipulation (genetic engineering) of crop plants to control pests by semiochems produced directly by the plant. Thus, nicotine has been found to be a defensive chemical for *Nicotiana sylvestris* against the larvae of herbivore *Manduca sexta* using genetically engineered plants (Voelckel *et al*., 2001). The feeding of this herbivore also induces the antimicrobial sesquiterpene capsidiol in the roots (Bohlmann *et al*., 2002). In wild tobacco, *Nicotiana attenuata*, the feeding of this herbivore also induces ethylene release, release of mono- and sesquiterpenes, a jasmonate burst, defence-related messenger RNAs (mRNAs), and a suppression of nicotine accumulation, as ascertained through cloning techniques (Winz and Baldwin, 2001). The sesquiterpenes appeared to act as antagonists to lepidopteran oviposition. Sagebush plants that were clipped or suffered damage in the field released a pulse of jasmonate that acted as a volatile signal that induced better grasshopper and cutworm resistance in neighbouring undamaged wild tobacco (Karban *et al*., 2000). This was the first demonstration of communication between two different species of plants.

Reviews are available on how plants respond to insect herbivores, including at the genetic level (Baldwin and Preston, 1999; Karban and Baldwin, 1997; Forbey and Foley, 2009; Sotka *et al*., 2009). Plant defence mechanisms require differential gene expression in signalling, sensory and metabolic pathways. Fatty acid semiochems (e.g. oxylipins synthesised from linolenic acid released in critical amounts from membranes by lipases) regulate expression of defence-related plant genes and are central to most wound-mediated plant responses. Phospholipid–amino acid conjugates found in insect gut, saliva or 'regurgitant' elicit emission of volatiles from plants that attract parasitoids like wasps that kill the insect pests. Neurotransmitters such as glutamate are known to be produced by plants, and have been postulated to be used in defence against animal herbivores (Baldwin and Preston, 1999). A glutamate receptor site in the genome of mustard seed (*Arabidopsis thaliana*) is thought to be involved in the normal sensing/response process, for example, communicating the presence of light, modulating light-dependent processes and regulating carbon and nitrogen metabolism (Kang and Turano, 2003) rather than being simply of purely defensive importance.

The recent research area of ecometabolomics, which aims to analyse the metabolome, the total number of metabolites and its shifts in response to environmental changes may allow predictive biomarkers of climate change to be found (Sardans *et al*., 2011). The field unites the fields of heat stress, cold hardiness, cold stress, nutrient and water deficiency, salinity stress, pollution stress and herbivore stress via the analysis of plant biomarkers like terpenes, ethylene and carbon dioxide that are known to be related to climate change.

The Future

The limitless potential of chemistry, biochemistry and genetics to describe biological mechanisms will undoubtedly be used in such areas as recognition of species of the same kind as well as of different kind; the interaction of physical factors of the environment with organisms and their adaptations; communication between injured and noninjured members of a species and the further investigation of interactions of organisms with other species. The genetic underpinnings of the chemical and biochemical changes, the genetic basis of signalling processes and how genes facilitate interactions will be areas of exciting discovery. Indeed the new RNA interference gene silencing/targeting technology has been used to characterise the genetic underpinnings of the behavioural ecology of the *Polistes* paper wasp, a sentinel insect for social evolution and behavioural ecology (Hunt *et al*., 2011).

Analyses of DNA sequences from cultivated microorganisms have revealed genome-wide, taxa-specific nucleotide compositional characteristics, referred to as genome signatures, currently in the form of tetranucleotide frequencies. However, little is known regarding the distribution of genome signatures in natural microbial communities or the extent to which environmental factors shape them (Dick *et al*., 2009). Shared environmental pressures and interactions among coevolving organisms did not obscure genome signatures in acid mine drainage microbial communities. Thus, genome signatures can be used to assign sequence fragments to populations, an essential prerequisite if metagenomics is to provide ecological and biochemical insights into the functioning of microbial communities.

The absolute dependence of ecological biochemistry on progress in analytical chemistry and biochemical method development and instrumentation must continue, especially now that macromolecular and genetic aspects are increasingly being investigated. The effects of mixtures also require such techniques to elucidate biological synergisms and antagonisms. **See also**: Chemical Ecology; Cyanogenesis in Higher Plants and Animals; DNA Profiling in Ecology; Metabolite Profiling in Plants; Molecular Ecology; Mutualism Among Free-living Species; Pheromones in Vertebrates; Phylogeny Based on 16S rRNA/DNA; Plant Defences against Fungal Attack: Biochemistry; Plant Defences against Herbivore Attack; Plant Gene Mapping Techniques; Plant Virus Movement and the Impact of RNA Silencing; Plant Virus Silencing Suppressors and RNA Silencing in Plants; Plant Volatiles; Predator-induced Plasticity; RNA Silencing and its Suppressors in the Plant-virus Interplay; mRNA Splicing: Role of snRNAs; Secondary Metabolites: Deterring Herbivores

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