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INVITED REVIEW

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Recent advances in chemical ecology: complex interactions mediated by molecules

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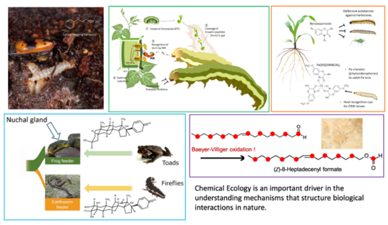
ABSTRACT

Chemical ecology is the highly interdisciplinary study of biochemicals that mediate the behavior of organisms and the regulation of physiological changes that alter intraspecific and/or interspecific interactions. Significant advances are often achieved through the collaboration of chemists and biologists working to understand organismal survival strategies with an eye on the development of targeted technologies for controlling agricultural, forestry, medical, and veterinary pests in a sustainable world. We highlight recent advances in chemical ecology from multiple viewpoints and discuss future prospects for applications.

Graphical Abstract



Chemical ecology is an important driver in the understanding mechanisms that structure biological interactions in nature.

Keywords: insect-animal interactions, insect-plant interactions, natural products chemistry, pest control, pheromones

Environmental change is certain to impact the abundance of individual species; however, complex ecosystems are likely to persist with a large number of species present. Biodiversity contributes to ecosystem stability and its importance is now widely accepted. Diverse species establish very different types of relationships, which span predator-prey interactions to mutualisms. Within a range of relationships and outcomes, it is important to study diverse interactions between organisms in food webs as well as biodiversity. Estimates of global food requirements in the year 2050, for a population of 9.7 billion people, project the need for a doubling in total crop production (Alexandratos and Bruinsma 2012; United Nations 2019). With an insufficient surplus of remaining available arable land on earth, a doubling of current crop yields is required. The understanding, control,

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and utilization of beneficial biological interactions in agricultural ecosystems are essential tools required to meet the demand for increased crop yields while reducing negative ecosystem and environment impacts.

The field chemical ecology has continued to expand from the 1960s as an increasingly interdisciplinary field seeking to understand the chemical language and basis of interactions between all organisms including insects, plants, animals, and microorganisms in terrestrial and aquatic ecosystems. Initially, the discipline focused solely on chemistry and ecology with an orientation toward the development of technologies for the control of agricultural and forestry pests. More broadly, basic chemical ecology research sought to understanding of the relationships between organisms mediated by chemicals in natural ecosystems. In the modern genomic era, chemical ecology now includes not only the chemical identification of signal molecules, but the more rapid elucidation of biosynthetic pathways, release and detection mechanisms, signal transduction pathways, neuroendocrine-mediated behaviors, and even developmental responses regulated. As a hybrid discipline, chemical ecology is an important driver in the understanding mechanisms that structure biological interactions in food web networks and provides a means of controlling interactions in agricultural ecosystems for sustainable food production.

In this review, the authors survey recent Chemical Ecology progress in (a) intraspecific interactions in insects and animals, (b) insect-plant interactions, and (c) insect-animal interactions. We place a particular focus on the interactions between organisms from both physiological and behavioral perspectives. The complex process of evolution in diverse species has resulted in diverse signals, communication, and perception systems that now provide increasing opportunities to harness the chemical languages for technological innovations in agriculture, public health, and related fields.

Intraspecific interactions in insects and animals

Small molecule chemistry is the basis and foundation of many communication mechanisms among insects. Chemicals involved in signaling between conspecific organisms, and affecting behavior modification, are termed pheromones. Pheromones are further divided into two distinct types of pheromones, releasers, and primers. Releaser pheromones initiate immediate behavioral responses in insects upon reception, while primer pheromones cause physiological changes in insects and animals that ultimately result in a behavior response. As releaser pheromones, many sex, alarm, aggregation, and trail pheromones have been identified in insects. Sex pheromones commonly specific to insect species and thus pheromones chemistry is commensurately diverse. Furthermore, elucidations of absolute configuration of pheromones with enantioselective synthesis have revealed the diversity of pheromonal communications in the natural world. Dr Kenji Mori significantly contributed to our understanding of the stereochemistry-bioactivity relationships among insect pheromones and has considered over 140 chiral pheromones (Mori 2007, 2014).

Recently, Takata *et al.* (2019) have reported a newly categorized pheromone, a provisioning pheromone, in the Burying beetle, *Nicropkorus quadripunctatus* (Coleoptera: Silphidae). Parent-offspring communication is essential to family process in many animals. Burying beetles exhibit complicated parental care, including offspring provisioning (Figure 1) (Eggert and Muller 1997; Scott 1998). Burying beetles breed on the carcasses of small vertebrates, and both of male and female parents feed predigested carrion to each larva via regurgitation. Larvae beg for parental provisioning by waiving their legs toward the mouthparts of the parents while raising their heads (Rauter and Moore 1999; Smiseth *et al.* 2003). Takata *et al.* (2019) have detected 2-phenoxyethanol in regurgitants derived from provisioning females, and also demonstrated that authentic 2-phenoxyethanol induced begging behavior from larvae. From these results, they identified 2-phenoxyethanol as a provisioning pheromone and concluded that the burying beetle, *N. quadripunctatus* inform their offspring of their preparation for provisioning by emitting a provisioning pheromone. It is well known that 2-phenoxyethanol shows germicide activity. So, these results strongly suggest that 2-phenoxyethanol serves primarily as a chemical germicide to suppress microbial growth in foods for offspring and has secondarily evolved as a provisioning pheromone. Furthermore, the identification of provisioning pheromones allowed the scientists to artificially manipulate larval begging behavior using an authentic compound, and to evaluate the cost of the begging behavior for the first time in an invertebrate. The interaction between parental feeding behavior and offspring begging is an important communication mechanism that regulates the food supply to offspring, and reducing the cost of superfluous begging is beneficial to both parent and offspring.

Figure 1. Female burying beetle provisioning larvae and the structure of 2-phenoxyethanol, which elicits begging behavior from their offspring. (The picture credit is: Mamoru Takata, Kyoto University; Yuki Mitaka, Kyoto Institute Technology).

In contrast to insects, primates have more developed visual communication systems; however, recently a putative sex pheromone have been found in the ring-tailed lemurs (*Lemur catta*) of Madagascar (Shirasu *et al.* 2020). Male lemurs rub their tail to the antebrachial glands located on the inside of their wrist and spread the glandular secretions by waiving the tail during breeding season. The amount of 3 aldehydes (dodecanal, 12-methyltridecanal, and tetradecanal) in the secretions increase during breeding season and female lemurs are attracted to the aldehyde mixture, specifically during the breeding season. To

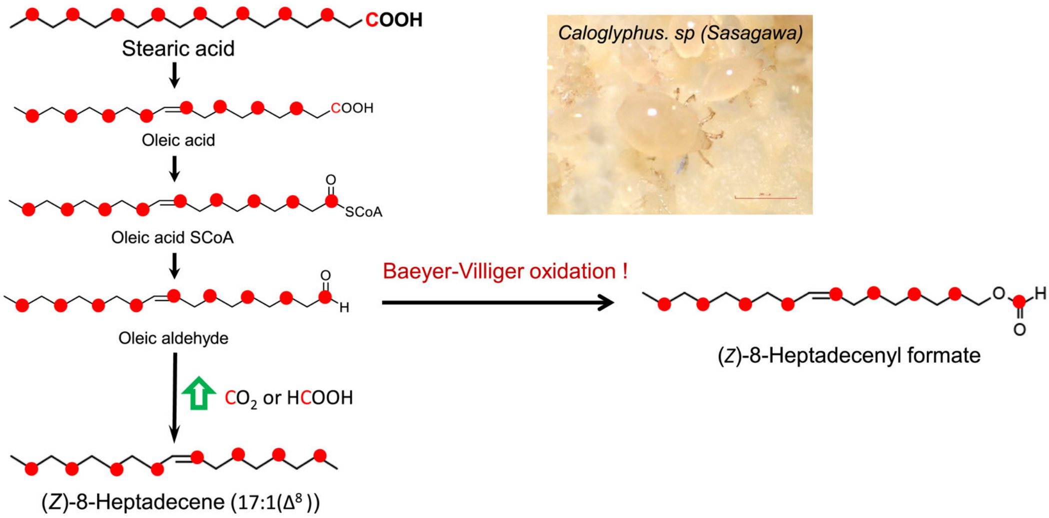

Figure 2. Biosynthesis of odd-numbered hydrocarbons and aliphatic formates in astigmatid mites.

understand the processes responsible for the evolution of chemically mediated systems not only in insects but in animals, we have to recognize how natural selections acts on interacting organisms.

Over the past 60 years, numerous pheromone studies have been done on insects, specifically identification of moth sex pheromones for the purpose of pest control. The discoveries shown above demonstrate that pheromone research is still fascinating to us and has the potential for further achievements.

Recent advances in semiochemical biosynthesis include newly established mechanisms of hydrocarbon and formate ester production in invertebrates. In insects, long chain hydrocarbons play important roles to prevent desiccation and serve in chemical communications (Gibbs 1998; Howard and Blomquist 2005). Although hydrocarbon biosynthesis has been established from fatty acids, involving a complex network of fatty acid biosynthesis, elongases, and desaturases, leading to long-chain acyl-CoA thioesters, which are converted to aldehydes, the final step from fatty aldehydes to hydrocarbons has remained mysterious. Qui *et al.* (2012), however, show that *Drosophila melanogaster* use a CYP4G family cytochrome P450 enzyme to oxidatively produce hydrocarbons from aldehydes with release of CO_2. Unlike cyanobacteria that make alkenes from aldehydes with nonhem diiron decarbonylases (Schirmer *et al.* 2010; Das *et al.* 2011; Eser *et al.* 2011; Li *et al.* 2011; Warui *et al.* 2011), a recombinant fusion protein of house fly CYP4G with P450 reductase catalyzes aldehyde decarbonylation by a radical mechanism. Hydrocarbons are produced by many organisms, including bacteria, algae, plants, and each appear to use a different mechanism for hydrocarbon production. These and related pioneering studies identify diverse mechanisms and genes useful in hydrocarbon production. In addition to creating molecular and enzyme targets for controlling insect pests, these genes may also be applied for biofuel production in algae and plants. Similar to this reaction shown above, Shimizu *et al.* (2017) proposed a novel biosynthetic pathway of aliphatic formates via a Baeyer-Villiger oxidation present in astigamatid mites (Figure 2). (Z, Z)-8,11-Heptadecadienyl formate (8,11-F17) and (Z)-8-heptadecenyl formate (8-F17) are rarely encountered natural products, which are abundant in an acarid mite (*Sancassania* sp.) (Acari: Acaridae). Several studies have shown that lipid components, such as formates and hydrocarbons, have the potential to be used for mite control (Skelton *et al.* 2010; Steidle *et al.* 2014). When ^{13}C-labeled acetic acid was administrated to the acarid mites, odd-numbered carbon atoms of linolenic and oleic acids (1,3,5,7,9,11,13,15,17-positions) are labeled through elongation and desaturation reactions in a regular manner. On the other hand, even-numbered carbon atoms of 8,11-F17 and 8-F17 (2,4,6,8,10,12,14,16-positions) are labeled with ^{13}C, with the labeled carbonyl carbon of formate. These results clearly revealed that the carbonyl carbon of the formates is originally derived from the C-1 position of the fatty acids with the insertion of an oxygen atom between the carbonyl group and carbon chain. Empirical data strongly supported that a mechanism whereby formates are generated via a Baeyer-Villiger oxidation of aldehydes as direct precursors. The enzyme(s) that catalyzes this reaction is yet to be identified in animals, microbes, and plants. In the near future, the demonstrated discovery of a Baeyer-Villiger monooxygenase capable of converting aliphatic aldehydes to formates would enable a facile dehomologation method in organic synthesis to provide molecules that had never accessed easily.

Insect-plant interactions

Plants produce extremely diverse specialized metabolites as a chemical arsenal for defense against insect herbivores. Common broad categories and classes encountered include alkaloids, terpenoids, phenylpropanoids, flavonoids, and nonprotein amino acids. Plant chemical defenses are often divided into 2 types of production strategies, constitutive and inducible defenses (Tumlinson *et al.* 1993; Wittstock and Gershenzon 2002; Hilker and Meiners 2006). In the case of constitutive defense production, healthy and unchallenged plants synthesize and store defensives that suppress attack from diverse organisms and also serve to negatively affect the growth, development, and survival of herbivores. The resource allocation of constitutive defenses is often viewed as costly for plants, but reduces the level of herbivory and severity of damage. Plants also deploy inducible defenses that are only produced after biotic attack. To overcome the chemical defenses of plants, many herbivorous insects have

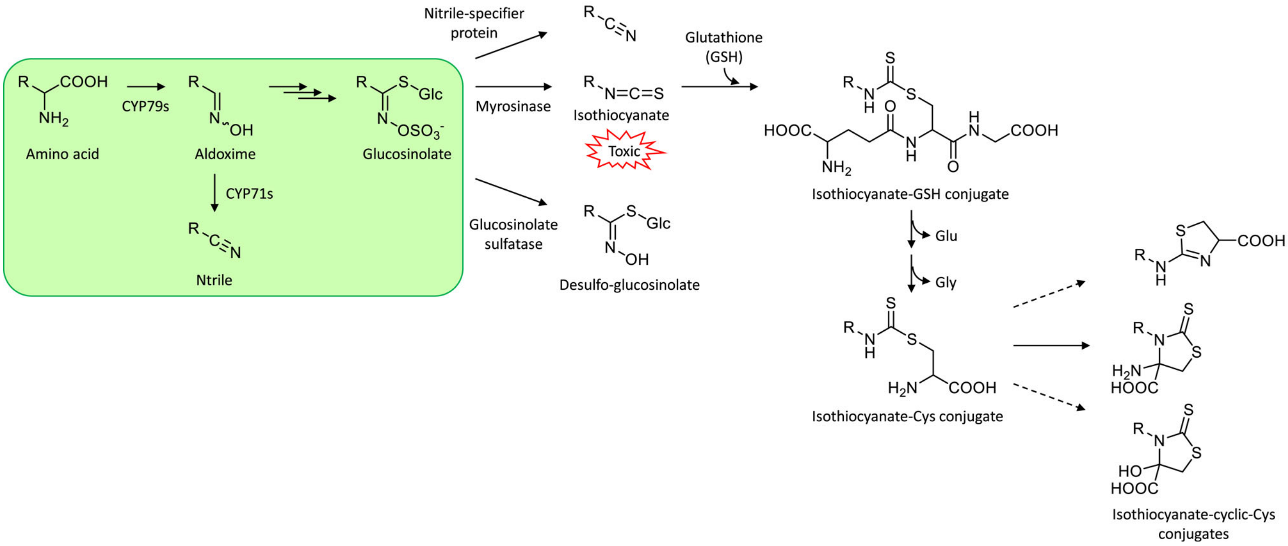

Figure 3. Biosynthetic pathway of glucosinolate and nitrile from amino acid in plants, and multiple detoxification pathway of glucosinolate-myrosinase defense system in insects.

evolved specialized detoxification mechanisms. Beyond detoxification, specialist herbivores often use plant defenses as specific chemical cues in host location and sequester plant derived toxins as defensive compounds against predators. On the other hand, plants possess complex innate immune systems that can respond to specific molecular patterns present in herbivore oral secretion and frass by amplifying wound-induced defenses against herbivorous insects. As described, the molecular investigation of insect-plant interactions is a significant subfield of chemical ecology that exists due to exceedingly complex layers of co-evolutionary arms races.

The glucosinolate-myrosinase defense system in Brassicaceae plants that liberates toxic isothiocyates is one of the most well-known plant chemical defenses (Fahey *et al.* 2001; Wittstock and Halkier 2002; Bone and Rossiter 2006). This system has fascinated researchers studying the ecological roles of isothiocyanates and elucidating the mechanisms of counter adaptation in Brassicaceae-feeding insects (Figure 3). One of the common detoxification mechanisms in insects is to avoid formation of toxic isothiocyanates by converting glucosinolate to desulfo-glucosinolate, which cannot be hydrolyzed by myrosinase. The nitrile-specifier protein found in insect gut can interact with myrosinase and then switch the glucosinolate hydrolysis products from isothiocyanates to less toxic nitriles (Wittstock *et al.* 2004). Another adaptation mechanism is to metabolize the resulting isothiocyanates during feeding with glutathione (Gloss *et al.* 2014). Recently, Beran *et al.* (2018) demonstrated that the adults of the cabbage stem flea beetle, *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae), partially sequester ingested glucosinolate, detoxify it by desulfation, and also deactivate isothiocyanates by conjugation with glutathione. The glucosinolate-glutathione conjugate is further metabolized to cyclic conjugates via the conserved mercapturic acid pathway. The glucosinolate-myrosinase defense system has long been a well-known plant chemical defense, but future studies will provide new findings that unveil the evolutionary arms race between potentially toxic plants and their herbivores.

As mentioned above, nitriles are known degradation products from glucosinolates. In contrast to this detoxification pathway, nitriles have recently been recognized as biosynthetic products from corresponding amino acids via aldoximes in diverse plant species (Figure 3; Irmisch *et al.* 2013; Noge and Tamogami 2013; Irmisch *et al.* 2014; Noge and Tamogami 2018). The production of nitriles are induced by herbivory, and they function either as repellents against herbivores or as chemical cues for natural enemies to locate prey. As described below, herbivory alters the physiological status of attacked plants. In nitrile biosynthesis, precursor amino acids and several other amino acids are also induced prior to nitrile production by herbivory or exogenous methyl jasmonate (Noge and Tamogami 2013; Noge and Tamogami 2018). Interestingly, it is reported that the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae), also synthesize phenylacetonitrile from L-phenylalanine (Wei *et al.* 2019). In this case, the nitrile function as an olfactory warning signal and a precursor of hydrogen cyanide, a toxin against predation by natural enemies, such as birds.

Benzoxazinoids, such as 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), are a class of indole-derived plant metabolites, and are widespread in grasses, including important cereal crops such as maize, wheat, and rye, as well as a few dicot species. Constitutively produced in seedlings, benzoxazinoids contribute to resistance against generalist herbivorous insects and fungi (Niemeyer 1988). The Western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is a specialist herbivorous insect to corn plants and one of the most devastating pests in North America. It is known that the root worm larvae can tolerate and sequester benzoxazinoids (Robert *et al.* 2012). Hu *et al.* (2018) show that the rootworm larvae use complexes between iron and benzoxazinoids to identify maize as a host, to forage within the maize roots, and to increase their growth (Figure 4). Because Fe-DIMBOA is only produced by a few other plant species, the complex is a reliable host cue for the larvae. Fe-DIMBOA is accepted as a substrate and directly improves Fe homeostasis for the larvae, causing improved larval performance. Furthermore, the larvae sequester the DIMBOA breakdown product MBOA for self-defense against entomopathogenic nematodes (Robert *et al.* 2017). This is the first report demonstrating a plant iron acquisition strategy mediated through benzoxazinoid defenses, which contribute to the Fe supply of maize plants, yet is further exploited by a specialist insect herbivore. This is a good

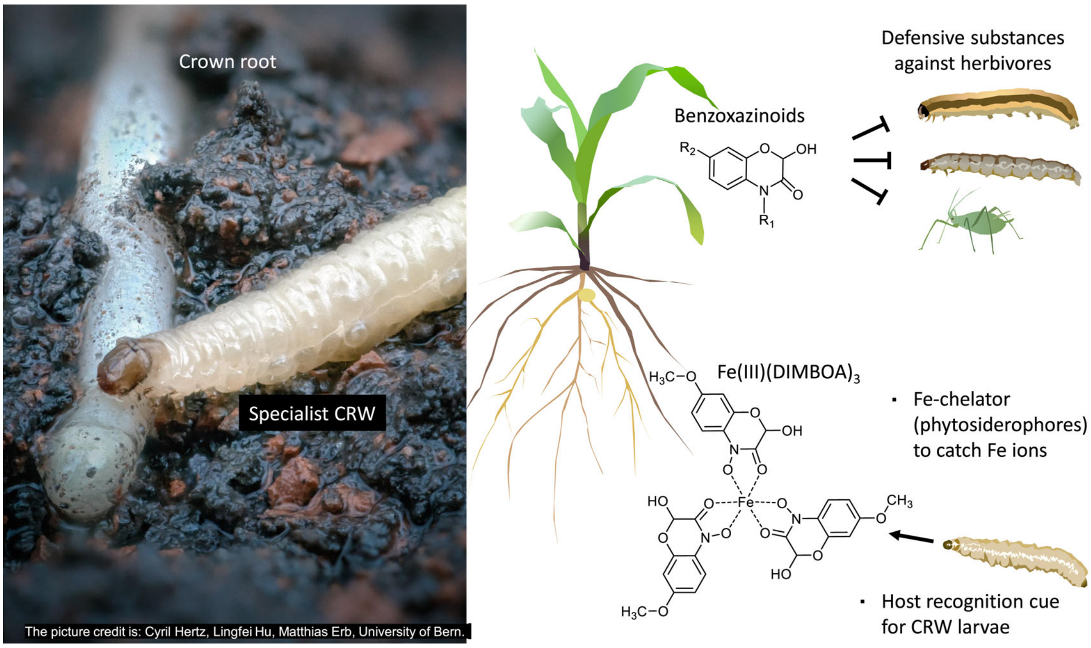

Figure 4. The western corn rootworm, a specialized maize herbivore, is fully resistant to benzoxazinoids and also uses complexes between Fe and benzoxizinoids to identify maize as a host recognition, to forage within the maize root system, and to increase their growth. (The picture credit is: Drs. Cyril Hertz, Lingfei Hu, Matthias Erb, University of Bern).

example revealing a new function of well-known compound, like DIMBOA.

As shown above, it is well known that insect species from almost all orders have evolved the ability to sequester plant toxins for their own defense. The larvae of the poplar leaf beetle, *Chrysomela populi* (Coleoptera: Chrysomelidae), sequester a plant glucoside, salicin, and subsequently convert the glucoside into the toxin, salicylaldehyde, stored in a defensive gland. Salicylaldehyde is released from the reservoir gland when the larvae get attacked (Pasteels *et al.* 1983; Kuhun *et al.* 2004; Michalski *et al.* 2008). The sequestration processes entails uptake, transfer, and concentration of specific phytochemicals into the hemolymph and specialized glands. Strauss *et al.* (2013) have identified a key transporter protein, *Cp*MRP, from *C. populi* larvae to accumulate salicin to their defensive gland. This transporter proteins belongs to a family of membrane proteins called ATP binding cassette (ABC) transporters, which help to shuttle chemicals out of cells or into cells using energy generated by the hydrolysis of ATP molecules (Chen *et al.* 1986; Ueda *et al.* 1987; Aller *et al.* 2009). In the defensive gland of the larvae, *Cp*MRP acts as a pacemaker for the irreversible shuttling of salicin from the hemolymph into defensive secretions. It is also demonstrated that *cpmrp*-silenced larvae are defenseless because they lack defensive secretions. However, transport studies in *Xenopus laevis* oocytes not only with a naturally sequestered host-plant glucoside of *C. populi* but with nonprecursor glucosides reveal that *Cp*MRP is not specific to salicin. Furthermore, Strauss *et al.* (2013) have identified transporter sequences highly similar to *Cp*MRP in the larval glands of other Chrysomelina species. This finding suggests that broad spectrum ABC transporters in the sequestration of plant metabolites are commonly present in *Chrysomelina* species. The described mechanism could explain why the leaf beetles (Coleoptera: Chrysomelidae) are able to utilize diverse host plants and sequester plant toxins as their own protective defensives.

In response to insect herbivory, many plants display inducible responses that serve to either directly inhibit pest growth or provide indirect defenses by promoting advantageous interactions with beneficial organisms (Howe and Jander 2008; Schmelz 2015; Erb and Reymond 2019). Elicitors present in herbivore oral secretions or frass have been demonstrated to positively regulate many biochemical changes (Figure 5). For example, corn plants (*Zea mays*) perceive insect herbivory through the detection of fatty acid amino acid conjugates elicitors present in oral secretions from larvae (Alborn *et al.* 1997; Truitt *et al.* 2004). Plant responses include the increased production and emission of volatiles that predators and parasitoids use as ecological signals in the search for prey and hosts. Since the discovery of fatty acid amino acid conjugates as insect derived elicitors, chloroplastic ATP synthase γ-subunit-derived peptide elicitors (inceptin), and a mixture of disulfooxy fatty acids (caeliferins) have been identified from oral secretions of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and of *Schistocera americana* (Orthoptera: Acrididae), respectively (Schmelz *et al.* 2006; Alborn *et al.* 2007). Interestingly, the fatty acid amino acid conjugates exhibit the widest range of volatile inducing activity in maize, soybean, and eggplant, although inceptin and caeliferins show the activity in cowpea and arabidopsis, respectively (Schmelz *et al.* 2009). These results strongly support the existence of specific receptor-ligand interactions mediating recognition. In 2019, Steinbrenner *et al.* (2019) identified a leucine-rich repeat receptor-like protein in bean plants (*Phaseolus* and *Vigna* species) as the inceptin receptor (INR) sufficient for elicitor-induced responses and enhanced defense against *Spodptera exigua* (Lepidoptera: Noctuidae). Dynamic and inducible plant defense responses to herbivores have been examined for nearly 50 years. Results by Steinbrenner *et al.* (2019) are first to demonstrate a plant cell surface receptor involved in the perception of precisely defined Herbivore Associated Molecular Patterns (HAMPs) present in insect oral secretions.

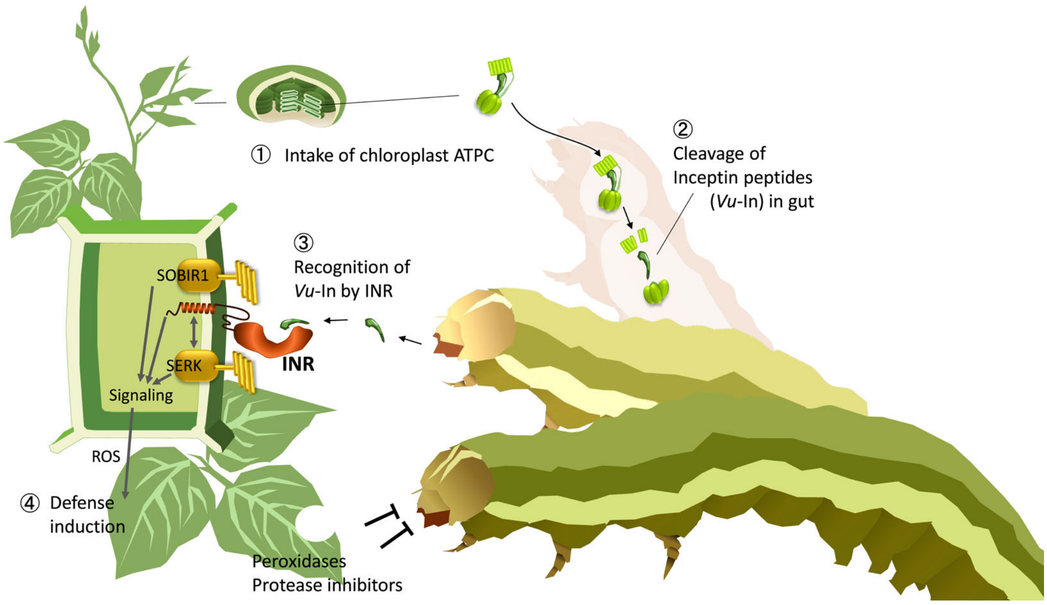

Figure 5. Conceptual scheme of chewing herbivore recognition and induced defense elicitation via inceptin receptor in cowpea. (original model kindly provided by Dr. EA Schmelz, University of California, San Diego, is partially modified).

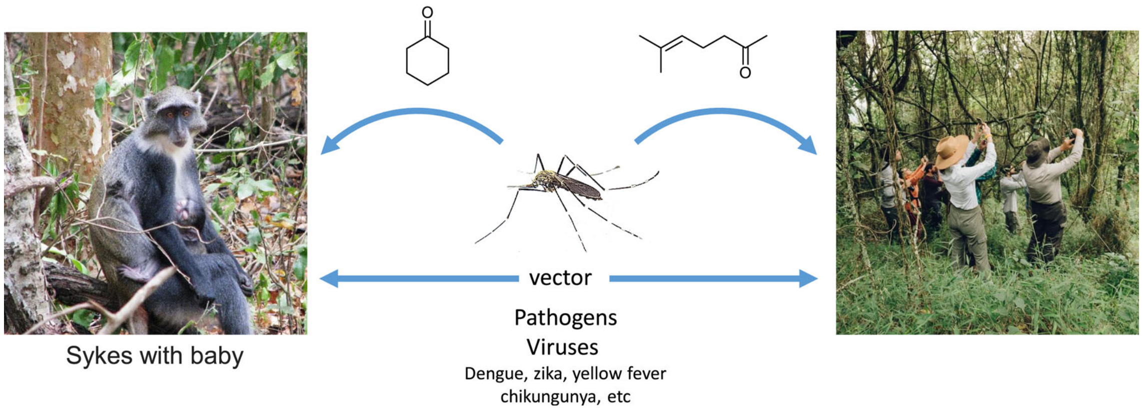

Figure 6. The *Aedes*-borne pathogens that cause dengue, zika, and yellow fever in humans originated from zoonotic cycle. Both nonhuman primates and humans are source of volatile odors that attract *Aedes* mosquitoes. (The picture credit of sykes with baby is: Dr. Baldwyn Torto, ICIPE).

The discovery expands the paradigm of cell-surface immune recognition established in plant-pathogen interactions to include plant perception and defense against the attackers from the Kingdom Animalia.

Insect-animal interactions

Insect-animal interactions appear less commonly studied than those of insect-plant and insect-insect interactions. However, understanding the chemical basis of insect-animal interactions remains important for sanitary pest control. We discuss recent advances in the development of lures and repellents of mosquito- and fly-borne viruses. We further highlight research using scent components from malaria-infected humans for effective diagnostic biomarkers. Finally we detail a novel finding that snakes (*Rhabdophis* spp) defend themselves with toxin derived from their insect prey, fireflies, while their ancestors obtain the related toxins from toads.

Arthropod-borne viruses (arboviruses) are a substantial threat to human and animal health world-wide. Many arboviruses are present in nature through the domestic and sylvatic cycles, where these viruses circulate. While rarely considered, very few infectious viruses are restricted to human hosts. The majority of viruses affecting humans are zoonotic agents that are maintained in enzootic cycles (Lloyd-Smith *et al.* 2009). For example, *Aedes* is a genus of mosquitoes originally found in tropical and subtropical countries, but has since spread to all continents except Antarctica. The *Aedes*-borne viruses that cause dengue, zika, and yellow fever in humans originated from zoonotic cycle. So, nonhuman primates (NHPs) are also important hosts for *Aedes* mosquitos. Interestingly, Tchouassi *et al.* (2019) show that *Aedes* mosquitos use cyclohexanone as a cue compound to detect NHP such as sykes, baboons, and vervets, whereas they use 6-methyl-5-hepten-2-one (sulcatone) to detect humans (Figure 6). Not only in the sylvatic but the domestic environment, CO_2-baited traps combined with either cyclohexanone or sulcatone increase capture rates compared to traps baited with CO_2 alone.

African trypanosomosis, responsible for persistent public health challenges in sub-Saharan Africa, is primarily

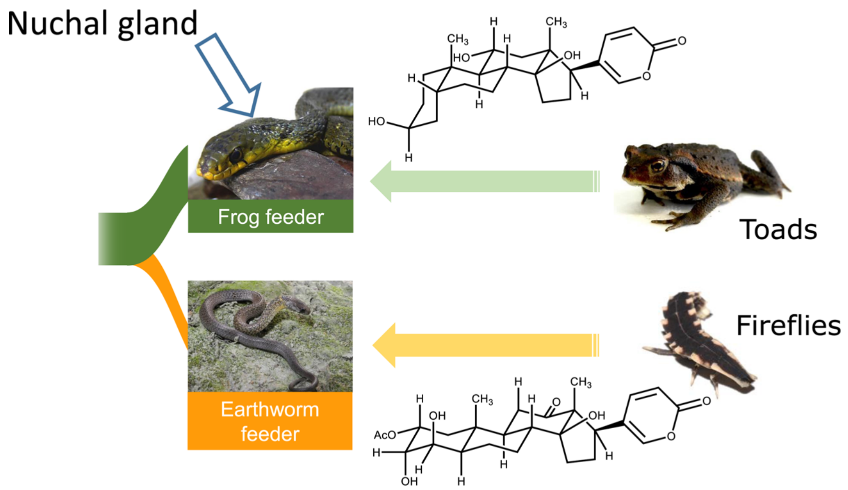

Figure 7. Evolutionally shift in diets of *Rhabdophis* species is accompanied by a novel source of sequestered bufadienolides, from toad to fireflies.

transmitted by tsetse flies, *Glossinia pallidipes* (Diptera: Glossinidae). Blood-feeding tsetse flies display different feeding preferences between animals. The preferences are due to different responses to animal skin odors. Thus the use of repellents from nonpreferred hosts could provide innovative tools for tsetse and African trypanosomosis control. In 2019, Olaide *et al.* (2019) have identified 7 active components; 6-methyl-5-hepten-2-one, acetophenone, geranylacetone, heptanal, octanal, nonanal, and decanal as repellant components from zebra skin odors. A seven-component blend in their natural ratio in zebra skin odor significantly reduced catches of *G. pallidipes* by 48.9%.

Despite long-standing control efforts, malaria remains among the world's deadliest diseases. Research on human and animal models has revealed that infection by malaria parasites causes changes in host odors that influence mosquito behaviors. It suggests that such changes of volatile compounds could provide reliable biomarkers for infection status. De Moraes *et al.* (2018) collected samples of skin volatiles from >400 primary-school children at 41 schools across 21 localities in Kenya between 2013 and 2016. To characterize the volatile signatures associated with infection status, De Moraes *et al.* (2018) used machine learning algorithms to demonstrate broad and consistent effects of malaria infection on human volatile profiles. Predictive of malaria infection status, important foot volatiles for model accuracy were 4-hydroxy-4-methylpentan-2-one, nonanal, toluene, and 2 unknown analytes. These findings suggest that volatile biomarkers have significant potential for the development of a reliable screening method for detecting malaria infections under field conditions.

Chemical ecology research can be applied not only to agriculture, but also to other fields such as public health. One of the fascinating points of chemical ecology research is that its high interdisciplinarity connects people in various fields as molecules connect the interactions between organisms.

Finally, we discuss recent results in shifting the sources of sequestered defensive toxins in snakes (*Rhabdophis* spp) as their diet changes. Snakes of the genus *Rhabdophis* store defensive toxins known as cardiotonic steroids, bufadienolides, in their dorsal skin, often limited to the neck (Figure 7). Following attack, the snakes release bufadienolide toxins against their predators. The ancestral diet of *Rhabdophis* snakes consists of anuran amphibians. *R. tigrinus* snakes sequester bufadienolides from toads consumed as prey (Hutchinson *et al.* 2007). Interestingly, members of a derived clade, the *Rhabdophis nuchalis* group, consume nontoxic earthworms as their primary prey, yet still sequester bufadienolides in their nucho-dorsal glands (Yoshida *et al.* 2020). Detailed chemical analyses reveal that bufadienolides sequestered in the *R. nuchalis* group, such as *R. pentasupralabialis*, possess bufadienolides similar to those as sequestered by *R. tigrinus* having a *cis*-A-B ring system. To date, bufadienolides with a *trans*-fused A-B ring system have only been identified from some firefly species (Eisner *et al.* 1978; Eisner *et al.* 1997; González *et al.* 1999; Fallon *et al.* 2018). From the nucho-dorsal glands of *R. pentasupralabialis*, Yoshida *et al.* (2020) identified a bufadienolide xyloside, which previously only known in fireflies. Furthermore, Yoshida *et al.* (2020) recovered larvae of lampyrine fireflies (*Diaphanes* sp.) from the stomach of *R. pentasupralabialis* snakes with gut contents in addition to earthworms. In separate laboratory feeding experiments, it was observed that some of *R. pentasupralabialis* consumed larvae of a related lampyrine firefly (*Pyrocoelia* sp.). Taken together, these chemical, feeding, and behavioral studies strongly indicated that *R. pentasupralabialis* includes lampyrinae firefly larvae in their natural diets and thus sequesters bufadienolides from fireflies instead of toads. This finding represents a remarkable evolutionary example where a shift from vertebrate to nonvertebrate prey maintains and continues to track biogenic dietary sources of useful defensive toxins.

Conclusions and perspectives

Currently, the global decline of insect populations has attracted worldwide attention (Hallmann *et al.* 2017). While often unnoticed, insects are the foundation of ecosystems that underlay human survival. The decline of insects and loss of ecosystem diversity/health negatively affects not only the global environment but also unavoidably us. In order to improve our understanding of the diversity of life on Earth and to develop sustainable technologies, we need to uncover the chemical languages and molecular mechanisms driving biological interactions. The modern era enables chemically mediated processes to be discovered, understood, and utilized to achieve desired outcomes while minimizing our planetary footprint. The combination of exciting research opportunities that have the promise to address critical societal needs provide young researchers with a springboard for valuable directions to pursue.

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References

Alborn HT, Hansen TV, Jones TH *et al.* Disulfooxy fatty acids from the american bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. *Proc Natl Acad Sci USA* 2007;**104**:12976-81.

Alborn HT, Turlings TCH, Jones TH *et al.* An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 1997; **276**:945-9.

Alexandratos N, Bruinsma J. *World Agriculture Towards 2030/2050: The 2012 Revision*. Rome: FAO; 2012. (ESA Working paper No. 12-03).

Aller SG, Yu J, Ward A *et al.* Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 2009;**323**:1718-22.

Beren F, Sporer T, Paetz C *et al.* One pathway is not enough: The cabbage stem flea beetle *Psylliodes chrysocephala* uses multiple strategies to overcome the glucosinolate-myrosinase defense in its host plants. *Front Plant Sci* 2018;**9**:1754.

Bone AM, Rossiter JT. The enzymatic and chemically induced decomposition of glucosinolates. *Phytochemistry* 2006;**67**:1053-67.

Chen C, Chin JE, Ueda K *et al.* Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* 1986;**47**:381-9.

Das D, Eser BE, Han J *et al.* Oxygen-independent decarbonylation of aldehydes by cyanobacterial aldehyde decarbonylase: A new reaction of diiron enzymes. *Angew Chem Int Ed Eng* 2011;**31**:7148-52.

De Moraes CM, Wanjiku C, Stanczyk NM *et al.* Volatile biomarkers of symptomatic and asymptomatic malaria infection in humans. *Proc Natl Acad Sci USA* 2018;**115**:5780-5.

Eggert AK, Muller JK. Biparental care and social evolution in burying beetles: lessons from the leader. In:Choe JC, Crespi BJ, editors. *The Evolution of Social Behavior in Insects and Arachnids*. Cambridge, UK: Cambridge University Press; 1997.

Eisner T, Goetz MA, Hill DE *et al.* Firefly “femmes fatales” acquire defensive steroids (lucibufagins) from their firefly prey. *Proc Natl Acad Sci USA* 1997;**94**:9723-8.

Eisner T, Wiemer DF, Haynes LW *et al.* Lucibufagins: Defensive steroids from the fireflies *Photinus ignitus* and *P. marginellus* (Coleoptera: Lampyridae). *Proc Natl Acad Sci USA* 1978;**75**:905-8.

Erb M, Reymond P. Molecular interactions between plants and insect herbivores. *Annu Rev Plant Biol* 2019;**70**:527-57.

Eser BE, Das D, Han J *et al.* Oxygen-independent alkane formation by non-heme iron-dependent cyanobacterial aldehyde decarbonylase: Investigation of kinetics and requirement for an external electron donor. *Biochemistry* 2011;**50**: 10743-50.

Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 2001;**56**:5-51.

Fallon TR, Lower SE, Chang CH *et al.* Firefly genomes illuminate parallel origins of bioluminescence in beetles. *eLife* 2018;**7**:71-146.

Gibbs AG. Water-proofing properties of cuticular lipids. *Am Zool* 1998;**38**:471-82.

Gloss AD, Vassão DG, Hailey AL *et al.* Evolution in an ancient detoxification pathway is coupled with a transition to herbivory in the Drosophilidae. *Mol Biol Evol* 2014;**31**: 2441-56.

González A, Schroeder FC, Attygalle AB *et al.* Metabolic transformations of acquired lucibufagins by firefly “femmes fatales.” *Chemoecology* 1999;**9**:105-12.

Hallmann CA, Sorg M, Jongejans E *et al.* More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One* 2017;**12**:e0185809.

Hilker M, Meiners T. Early herbivore alert: Insect eggs induce plant defense. *J Chem Ecol* 2006;**32**:1379-97.

Howard RW, Blomquist GJ. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu Rev Entomol* 2005;**50**:371-93.

Howe GA, Jander G. Plant immunity to insect herbivores. *Annu Rev Plant Biol* 2008;**59**:41-66.

Hu L, Mateo P, Ye M *et al.* Plant iron acquisition strategy exploited by an insect herbivore. *Science* 2018;**361**:694-7.

Hutchinson DA, Mori A, Savitzky AH *et al.* Dietary sequestration of defensive steroids in nuchal glands of the Asian snake *Rhabdophis tigrinus*. *Proc Natl Acad Sci USA* 2007;**104**:2265-70.

Irmisch S, Clavijo McCormick A, Boeckler GA *et al.* Two herbivore-induced cytochrome P450 enzymes CYP79D6 and CYP79D7 catalyze the formation of volatile aldoximes involved in poplar defense. *Plant Cell* 2013;**25**:4737-54.

Irmisch S, Clavijo McCormick A, Gunther J *et al.* Herbivore-induced poplar cytochrome P450 enzymes of the CYP71 family convert aldoximes to nitriles which repel a generalist caterpillar. *Plant J* 2014;**80**:1095-107.

Kuhun J, Pettersoon EM, Feld BK *et al.* Selective transport systems mediate sequestration of plant glucosides in leaf beetles: a molecular basis for adaptation and evolution. *Proc Natl Acad Sci USA* 2004;**108**:13808-13.

Li N, Nørgaard H, Warui DM *et al.* Conversion of fatty aldehydes to alka(e)nes and formate by a cyanobacterial aldehyde decarbonylase: Cryptic redox by an unusual dimetal oxygenase. *J Am Chem Soc* 2011;**133**:6158-61.

Lloyd-Smith JO, George D, Pepin KM *et al.* Epidemic dynamics at the human-animal interface. *Science* 2009;**326**:1362-7.

Michalski C, Mohagheghi H, Nimtz M *et al.* Salicyl alcohol oxidase of the chemical defense secretion of two chrysomelid leaf beetles -molecular and functional characterization of two new members of the glucose-methanol-choline oxidoreductase gene family-. *J Biol Chem* 2008;**283**:19219-28.

Mori K. Significance of chirality in pheromone science. *Bioorg Med Chem* 2007;**15**:7505-23.

Mori K. Stereochemical studies on pheromonal communications. *Proc Jpn Acad Ser B* 2014;**90**:373-88.

Niemeyer NM. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defense chemicals in the Gramineae. *Phytochemistry* 1988;**27**:3349-58.

Noge K, Tamogami S. Herbivore-induced phenylacetonitrile is biosynthesized from de novo-synthesized L-phenylalanine in the giant knotweed, *Fallopia sachalinensis*. *FEBS Lett* 2013;**587**:1811-7.

Noge K, Tamogami S. Isovaleronitrile co-induced with its precursor, L-leucine, by herbivory in the common evening primrose stimulates foraging behavior of the predatory blue shield bug. *Biosci Biotechnol Biochem* 2018;**82**:395-406.

Olaide OY, Tchouassi DP, Yusuf AA *et al*. Zebra skin odor repels the savannah tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). *PLoS Negl Trop Dis* 2019;**13**:e0007460.

Pasteels JM, Rowell-Rahier M, Braekman JC *et al*. Salicin from host plant as precursor of salicylaldehyde in defensive secretion of Chrysomeline larvae. *Physiological Entomol* 1983;**8**:307-14.

Qiu Y, Tittiger C, Wicker-Thomas C *et al*. An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. *Proc Natl Acad Sci USA* 2012;**109**:14858-63.

Rauter CM, Moore AJ. Do honest signaling models of offspring solicitation apply to insects? *Proc R Soc Lond B* 1999;**266**:1691-6.

Robert CAM, Veyrat N, Glauser G *et al*. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecol Lett* 2012;**15**:55-64.

Robert CAM, Zhang X, Machado RAR *et al*. Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *eLife* 2017;**6**:e29307.

Schirmer A, Rude MA, Li X *et al*. Microbial biosynthesis of alkanes. *Science* 2010;**329**:559-62.

Schmelz EA, Carroll MJ, LeClere S *et al*. Fragments of ATP synthase mediate plant perception of insect attack. *Proc Natl Acad Sci USA* 2006;**103**:8894-9.

Schmelz EA, Engelberth J, Alborn HT *et al*. Phytohormone-based activity mapping of insect herbivore-produced elicitors. *Proc Natl Acad Sci USA* 2009;**106**:653-7.

Schmelz EA. Impacts of insect oral secretions on defoliation-induced plant defense. *Insect Sci* 2015;**9**:7-15.

Scott MP. The ecology and behavior of burying beetles. *Annu Rev Entomol* 1998;**43**:595-618.

Shimizu N, Sakata D, Schmelz EA *et al*. Biosynthetic pathway of aliphatic formates via a Baeyer–Villiger oxidation in mechanism present in astigmatid mites. *Proc Natl Acad Sci USA* 2017;**114**:2616-21.

Shirasu M, Ito S, Itoigawa A *et al*. Key male glandular odorants attracting female ring-tailed lemurs. *Curr Biol* 2020;**30**:2131-8.

Skelton AC, Cameron MM, Pickett JA *et al*. Identification of neryl formate as the airborne aggregation pheromone for the American house dust mite and the European housedust mite (Acari: Epidermoptidae). *J Med Entomol* 2010;**47**:798–804.

Smiseth PT, Darwell CT, Moore AJ. Partial begging: an empirical model for the early evolution of offspring signaling. *Proc R Soc Lond. B* 2003;**270**:1773-7.

Steidle JLM, Barcari E, Hradecky M *et al*. Pheromonal communication in the European house dust mite, *Dermatophagoides pteronyssinus*. *Insects* 2014;**5**:639-50.

Steinbrenner AD, Munoz-Amatriain M, Venegas JMA *et al*. A receptor for herbivore-associated molecular patterns mediates plant immunity. bioRχiv. 2019, DOI:http://dx.doi.org/10.1101/679803.

Strauss AS, Peters S, Boland W *et al*. ABC transporter functions as a pacemaker for sequestration of plant glucosides in leaf beetles. *eLife* 2013;**2**:e01096.

Takata M, Mitaka Y, Steiger S *et al*. A parental volatile pheromone triggers offspring begging in a burying beetle. *iSceince* 2019;**19**:1256-64.

Tchouassi DP, Jacob JW, Ogola O *et al*. *Aedes* vector–host olfactory interactions in sylvatic and domestic dengue transmission environments. *Proc R Soc B* 2019;**286**:2136

Truitt CL, Wei H-X, Paré PW. A plasma membrane protein from *Zea mays* binds with the herbivore elicitor volicitin. *Plant Cell* 2004;**16**:523-32.

Tumlinson JH, Turlings TCJ, Lewis WJ. Semiochemically mediated foraging behavior in beneficial parasitic insects. *Arch Insect Biochem Physiol* 1993;**22**:385-91.

Ueda K, Pastan I, Gottesman MM. Isolation and sequence of the promotor region of the human multidrug-resistance (P-glycoprotein) gene. *J Biol Chem* 1987;**262**:17432-6.

United Nations, *Department of Economic and Social Affairs, Population Division*. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423); 2019.

Warui DM, Ning Li N, Nørgaard H *et al*. Detection of formate, rather than carbon monoxide, as the stoichiometric coproduct in conversion of fatty aldehydes to alkanes by a cyanobacterial aldehyde decarbonylase. *J Am Chem Soc* 2011;**133**:3316-9.

Wei J, Shao W, Cao M *et al*. Phenylacetonitrile in locusts facilitates an antipredator defense by acting as an olfactory aposematic signal and cyanide precursor. *Sci Adv* 2019;**5**:eaav5495.

Wittstock U, Agerbirk N, Stauber EJ *et al*. Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proc Natl Acad Sci USA* 2004;**101**:4859-64.

Wittstock U, Gershenzon J. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr Opin Plant Biol* 2002;**5**:300-7.

Wittstock U, Halkier BA. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci* 2002;**7**:263-70.

Yoshida T, Ujiie R, Savitzky AH *et al*. Dramatic dietary shift maintains sequestered toxins in chemically defended snakes. *Proc Natl Acad Sci USA* 2020;**117**:5964-9.