Annual Review of Entomology

Insect Sterol Nutrition: Physiological Mechanisms, Ecology, and Applications

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Annu. Rev. Entomol. 2020. 65:12.1–12.21

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-011019-025017

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Keywords

cholesterol, nutritional physiology, Niemann-Pick type C proteins, hormone receptor 96, sterol homeostasis, sterol auxotroph

Abstract

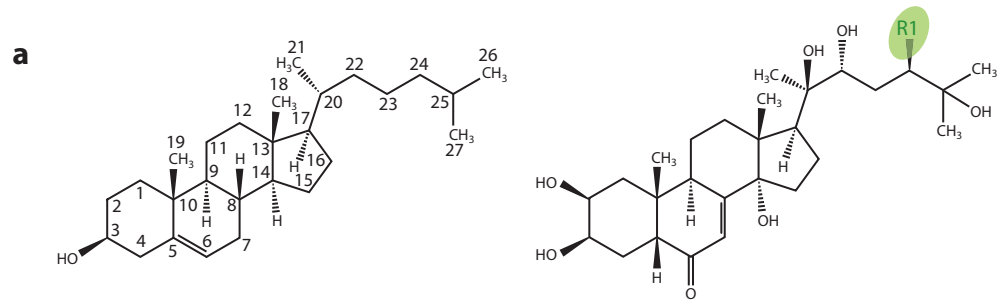
Insects, like all eukaryotes, require sterols for structural and metabolic purposes. However, insects, like all arthropods, cannot make sterols. Cholesterol is the dominant tissue sterol for most insects; insect herbivores produce cholesterol by metabolizing phytosterols, but not always with high efficiency. Many insects grow on a mixed-sterol diet, but this ability varies depending on the types and ratio of dietary sterols. Dietary sterol uptake, transport, and metabolism are regulated by several proteins and processes that are relatively conserved across eukaryotes. Sterol requirements also impact insect ecology and behavior. There is potential to exploit insect sterol requirements to (*a*) control insect pests in agricultural systems and (*b*) better understand sterol biology, including in humans. We suggest that future studies focus on the genetic mechanism of sterol metabolism and reverse transportation, characterizing sterol distribution and function at the cellular level, the role of bacterial symbionts in sterol metabolism, and interrupting sterol trafficking for pest control.

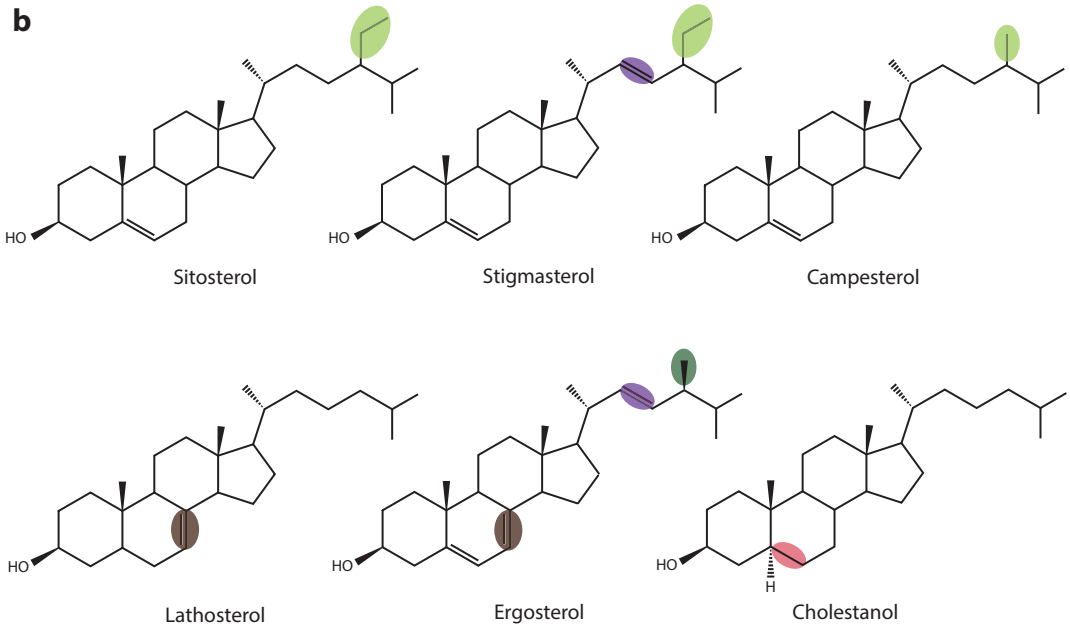
INTRODUCTION

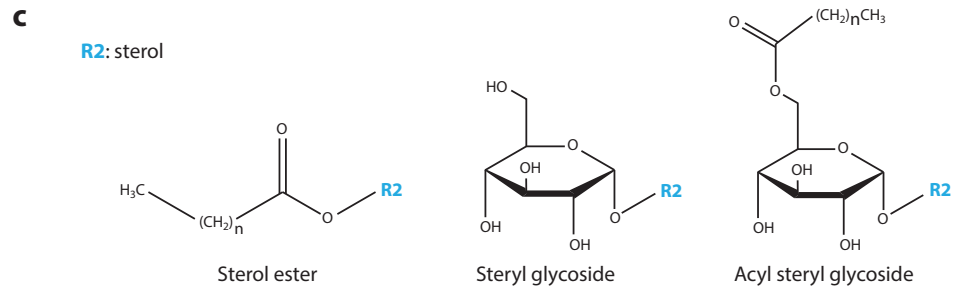
Insects, like all eukaryotes, use sterols in three critical ways: first, as structural components in the phospholipid bilayer of the membranes of cells and organelles (11, 25); second, as precursors to important steroid hormones (48, 89, 133); and third, as a molecule that regulates organismal growth and patterning via the Hedgehog (Hh) signaling pathway (29, 107). More recently, sterols have been associated with defense against pathogenic agents and parasitoids (23, 108). Cholesterol (**Figure 1**), the most-studied and best-understood sterol, was first discovered 261 years ago in human gallstones by the French doctor Francois Poulletuer de La Salle. It is an omnipresent lipid in animals, including insects, but typically occurs in small absolute amounts, which vary depending upon the species, size, and feeding biology (11). For example, sterols tend to occur at very low levels in aphids (e.g., 0.06 μg/mg in *Schizaphis graminum*), but at higher concentrations in grasshoppers (e.g., 1.6–2.5 μg/mg in *Schistocerca americana*) and caterpillars (e.g., 0.78–1.29 μg/mg in *Helicoverpa zea*) (8, 21, 69, 71).

The bulk of sterols in animals, including insects, is incorporated into the phospholipid bilayer of cells and organelles. Theoretically, sterols can account for up to half of the total lipid molecules in cellular membranes (2, 47). But how do sterols behave in lipid bilayers? Sterols are amphipathic molecules, meaning that they have hydrophilic (polar) and hydrophobic (nonpolar) parts. The polar part, a C3 hydroxyl group, orients outward from the bilayer and can form hydrogen bonds with the hydrophilic phosphate head of a phospholipid molecule. In contrast, the nonpolar portion is composed of a tetracyclic triterpenoid and an isooctyl hydrocarbon chain. This hydrophobic part of a sterol extends inwards and interacts with phospholipid fatty acid tails; more specifically, the tetracyclic structure and the side chain align adjacently to the fatty acid chain (60). In the bilayer, cholesterol and phospholipids interact to enhance mechanical coherence of the membrane by reducing fluidity (i.e., cholesterol acts as a stabilizer when surrounded by phospholipid molecules) and increasing rigidity (i.e., the ordering of the hydrocarbon chains). This has the effect of suppressing passive permeability, which improves the ability of a cell to control the movement of various molecules, especially polar ones, across the membrane and into the cell (98). There are also cholesterol-rich regions—termed lipid rafts—in the phospholipid bilayer. These lipid rafts have physical features that are quite distinct from those of the surrounding membrane landscape and are often associated with integral membrane proteins (e.g., transmembrane proteins). However, these sites tend to be enriched in sphingolipids (another major class of membrane lipids), rather than phospholipids (54, 153). Currently, our understanding of lipid raft distribution, structure, and function in cell membranes is relatively basic, especially in insects.

A relatively small amount of sterol is required for metabolic purposes, in particular for producing molting hormone. The details of insect molting have been reviewed thoroughly elsewhere (41, 82, 147), so we provide a quick overview with an emphasis on variation in molting hormone structure as a function of the sterol precursors being used. In most insects, 20-hydroxyecdysone (20E) is the major molting hormone (**Figure 1**), and cholesterol is the required precursor. Some insects, including plant-feeding heteropterans, leaf-cutting ants, and honeybees, use the ecdysteroid makisterone as their molting hormone (82, 93, 115, 134). These insects have lost the ability to dealkylate phytosterols. Instead, they directly convert campesterol into makisterone A (which has a methyl at the C24 position) or sitosterol into makisterone C (which has an ethyl at the C24 position) (**Figure 1**). Molting hormone variation tied to sterol use is also illustrated by the adaption of *Drosophila pachea*, a specialist on the senita cactus (*Lophocereus schottii*). This food resource is rich in lathosterol (**Figure 1**), so it serves as the precursor for the ecdysteroid that drives molting in *D. pachea*; this is in contrast to *D. pachea*'s close relative *Drosophila melanogaster*, which uses cholesterol. The exclusive dependence of *D. pachea* on lathosterol as the







(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Chemical structure of (*a*) cholesterol and steroid hormones, (*b*) sterols, and (*c*) sterol conjugates. Cholesterol is the most common sterol found in insects. 20-Hydroxyecdysone (20E) is the major steroid hormone in most insects; cholesterol is its precursor. Some insects use another group of steroid hormones, makisterone A or C; they differ from 20E in that they contain an additional methyl or ethyl group, respectively, at C24 (R1) (82). Sitosterol (24-ethylcholest-5-en-3β-ol), stigmasterol (24-ethylcholest-5,22-en-3β-ol), and campesterol (24-methylcholest-5-en-3β-ol) are common phytosterols. They differ structurally from cholesterol by a methyl or ethyl group (*light green*) in the side chain at C24 and/or a C22 double bond (*purple*); 24α-alkylcholesterol is often seen in evolutionarily derived plants (146). These phytosterols can be directly converted into steroid hormones, i.e., makisterone A or C. Lathosterol (5α-cholest-7-en-3β-ol) is a sterol produced in the senita cactus that *Drosophila pachea* uses to produce 20-hydroxyecdysone (85); it differs from cholesterol only by the shift of the double bond from C5 to C7 (*brown*). Ergosterol (5,7,22-ergostatrien-3β-ol) is a common fungal sterol that contains a beta-methyl group (*dark green*) at C24 [in contrast to the dominant alpha-alkyl sterols in plants (93)]; it also has two additional double bonds at C7 (*brown*) and C22 (*purple*). Cholestanol (5α-cholestan-3β-ol) is saturated at C5 (*pink*), in contrast to cholesterol. Sterol ester, steryl glycoside, and acyl steryl glycoside comprise the three major groups of conjugated sterols, with the 3-OH of sterols (R2) covalently bonded to fatty acids, sugars, and sugar esters, respectively (43).

precursor substrate is the result of four amino acid changes in the oxygenase-like protein encoded by a *neverland* gene (85).

Unlike yeasts and vertebrates, insects (and all other arthropods) are sterol-auxotrophs (11, 25). This inability to produce sterols likely exists because insects lack the enzyme-coding gene that converts farnesyl pyrophosphate to squalene (120, 139). The fact that insects have lost the ability to generate sterols de novo is puzzling. Perhaps there is an evolutionary advantage to sterol-auxotrophy given that (*a*) insects have innate oxygen-supply limitations as a function of their blind-ended tracheal respiratory system (56), and (*b*) cholesterol synthesis is extremely oxygen consuming and metabolically expensive. An inadequate oxygen supply could place a significant constraint on the synthesis of sterols, and this could reduce and/or delay insect growth (32, 37, 103, 118).

Since the last comprehensive review on insect sterol utilization—published 14 years ago (11)—several technological advances, including advanced gas and liquid chromatography, gene chips, high-throughput sequencing and screening, and cellular and molecular immunology and biochemistry, have been applied to better understand insect sterol nutrition and physiology. Our goal in this review is to provide an overview of the most recent advances concerning insect sterol nutrition, ranging from metabolism to homeostasis, physiological ecology, and the potential of exploiting insect sterol metabolic constraints to manage insect herbivore pests; in addition, we aim to more deeply explore sterol biology, including its implications for humans.

STEROL FORM, FUNCTION, AND METABOLISM

Sterol Use in Different Insects and Consequences of Feeding on Different Sterols

There is significant structural variation in sterols—more than 1,000 natural cholesterol derivatives have been identified from a broad range of organisms (102). However, most common sterols contain at least one double bond (C5), with additional double bonds sometimes occurring at other positions, including C7, C22, and/or C24. Cholesterol, phytosterols, and ergosterol are the major sterols in animals, plants, and fungi, respectively (**Figure 1**). Half of all insects eat plants, and 200 unique sterols have been recorded from different plant species. Additionally, most individual plant species contain multiple sterols; in some cases, more than 60 unique sterols have been identified (although most of these occur at nanogram levels) (14). For example, in hemp seed, 70% of the identified phytosterols comprise less than 2% of the total sterol profile (92). Sitosterol, campesterol, and stigmasterol are the most common phytosterols (92, 97), which mainly differ from cholesterol by a methyl or ethyl substituent at C24 (**Figure 1**). Moreover, sterols can occur

in free forms or as conjugates. In the latter case, the 3β-OH of sterols conjugates with fatty acids or carbohydrates (**Figure 1**) (43, 54, 67). There are also many sterol enantiomers. Theoretically, cholesterol has 256 enantiomers, but the only one found in organismal membranes is the 3R, 20R configuration—which has an equatorial C3 hydroxyl group (102). It has been suggested that the physicochemical properties of the proteins involved in sterol synthesis may determine the stereospecific formation of sterols (30, 51). Finally, stanols (saturated at C5; **Figure 1**) are a type of steroid similar to sterols. They are also found in plants, but usually only in small amounts (111). Insects reared on stanol-rich diets accumulate stanols in their tissues (69, 71). Most sterols have the general stereo-structure needed for function in cellular membrane, but variation in the type, amount, and ratio of different dietary sterols has species-specific effects on the rate and total growth of insects (5, 6, 25, 69).

Insects generally acquire sterols from two main sources: parental loading during oogenesis and food. The sterol content in embryos—typically more than half being conjugated to fatty acids such as palmitate, oleate, or stearate (54, 73)—derives mostly from maternal loading of sterols. This is particularly true for parthenogenic insects like aphids (19). However, some sterol also comes from membrane cholesterol originating from sperm (16, 45). This collective reservoir is usually enough to allow the first molt of *Drosophila* neonates reared on diets that lack dietary sterols (45, 140). Ultimately, insufficient cholesterol ingestion during larval stages can affect oogenesis and lead to reduced fecundity (9, 16, 28, 36, 55, 69, 70, 96, 113). There are, however, instances where fecundity can be recovered by supplementing with dietary cholesterol, as shown in the yellow fever mosquito, *Aedes aegypti* (130). This suggests that there may be a sterol threshold for oogenesis. Interestingly, despite the need for females to allocate a significant amount of cholesterol into embryos, *Manduca sexta* discharge about one-fourth of their total cholesterol in the meconium at emergence (73).

As immature insects grow and develop, dietary sterols are mostly allocated toward cellular membranes, although there is tissue-specific distribution of different sterols (e.g., cholesterol as the dominant membrane insert in central nervous system tissue), and development is severely impaired when sterol supply is interrupted (18, 19, 24, 25, 42, 68, 70, 73). Insect dietary sterol use studies published prior to 2003 were comprehensively summarized by Behmer & Nes (11). Since their review, additional studies on insect sterol use have been published, and we summarize those findings in **Supplemental Table S1**. Most insects can use cholesterol directly; examples of exceptions include one dipteran (*D. pachea*) and two lepidopteran species (*Homona coffearia* and *Crambus trisecta*). However, insect herbivores, unlike carnivorous insects, rarely encounter sufficient amounts of dietary cholesterol. Instead, they ingest (and metabolize) phytosterols that occur in plants in concentrations of ca. 1–3 mg/g dry weight. This amount is generally equal to or higher than sterol concentrations found in insect herbivores, with pollen sometimes being an exception (18, 19, 42, 61, 68, 70, 112).

The tissue profile of plant-feeding insects is mostly cholesterol given that many herbivorous insects readily convert ingested common phytosterols to cholesterol, especially lepidopteran species (**Supplemental Table S1**) (11, 69, 71). However, there are exceptions. Grasshoppers (Orthoptera: Acrididae) can only dealkylate sitosterol and campesterol (4, 6, 8), while heteropterans have completely lost this ability (127). Some insects can dealkylate but are not particularly efficient. Aphids generally convert sitosterol to cholesterol at a rate of approximately 40% (21), and, consistent with reports for other dipteran insects, *D. melanogaster* has a limited ability to metabolize dietary sterols (24). However, *D. melanogaster* is very good at using various cholesterol-like molecules, including common phytosterols, in lipid bilayers (25, 82, 88). The flexibility to use sterols other than cholesterol as membrane inserts can be highly beneficial for insects, especially for those that use ecdysone as their hormone. For such insects, a small amount of cholesterol must always be

available and spared for producing ecdysone (117). However, the ability of insects to use a mixture of sterols in their membranes varies from species to species (3). As stated above, *D. melanogaster* can tolerate a high proportion of noncholesterol sterols in their membranes. In contrast, grasshoppers cannot (4–6, 8, 24, 25). Lepidopteran insects are somewhere in between. They can use atypical sterols or steroids as membrane structure, but only up to a threshold (**Supplemental Table S1**) (70).

Recent Advances in Identifying Genes Involved in Insect Phytosterol Metabolism

The catalytic steps that insects use for converting phytosterols into cholesterol has been revealed by identifying sterol metabolites (125). However, much less is known about the genetic basis of sterol metabolism, especially dealkylation. Recently, a microsomal membrane-associated reductase primarily enriched in the gut of *Bombyx mori* has been shown to mediate the in vitro conversion of desmosterol, the last metabolite in the dealkylation, into cholesterol (27). This enzyme belongs to a flavin adenine dinucleotide–dependent oxidoreductase family, and the vertebrate orthologs catalyze the reduction of demosterol to cholesterol in the de novo cholesterol biosynthesis pathway (142). Additionally, around this time, Jing et al. (71) conducted the first genome-wide study on sterol metabolism in one caterpillar species, *H. zea*, and identified a number of genes potentially involved in dealkylation. Interestingly, lepidopteran insects can metabolize 3-keto-steroids into two diastereomers, 3α- and 3β-cholestanol, potentially by 3α-reductase and 3β-reductase, respectively. These two enzymes were previously described in the metabolism of 3-keto-ecdysone. The multiple functions of sterol-metabolizing enzymes hint at the complexity of the sterol metabolism network in insects (69, 148). Auchenorrhynchan insects can survive on sterol-poor xylem, and cholesterol is the major sterol in these insects (13, 67). Recently, a parallel genomic analysis on a planthopper species, *Nilaparvata lugens*, and its yeast-like symbiont revealed that the host genome encodes the four key enzymes to convert zymosterol—produced by the symbiont—into cholesterol. Additionally, it appears that a shared metabolic pathway—between the host and the fungal symbiont—directs metabolism of zymosterol to cholesterol. However, verifying this is challenging, as is often the case for research using omics-based approaches and technologies (35).

STEROL HOMEOSTASIS: CELLULAR UPTAKE AND TRANSPORT

All eukaryotes have and use sterols for physiological purposes, but sterol homeostasis mechanisms differ between arthropods and vertebrates. For example, in vertebrates, sterol regulatory element–binding proteins (SREBPs) sense cholesterol levels in the endoplasmic reticulum (ER) and subsequently coordinate cholesterol synthesis and cholesterol uptake machinery. In insects, and despite its name, SREBPs sense and regulate phospholipids (33, 65, 120). Nonetheless, all eukaryotes have mechanisms in place that regulate the flow of sterols into and out of cell and organelle membranes, as well as facilitating their intracellular transportation once inside a cell. Sterols can also be found in other aqueous milieu in eukaryotes, including the gut lumen and blood. In the insect gut lumen, free sterols combine with other free lipids to form soluble micelles that can be absorbed across the peritrophic membrane and into the lipid bilayer of enterocytes. In the aqueous hemolymph, free sterols are solubilized by specialized transport particles that shuttle hydrophobic sterols between different organs and tissues.

Cellular sterol concentrations in insects are tightly regulated (25), and when there is a surplus of cholesterol, it can be converted into a cholesterol ester or removed by cellular efflux (73, 122). The molecular mechanisms regulating this process have been studied extensively in recent years,

and we graphically summarize this process in **Figure 2**. Sterols enter into enterocytes mainly via NPC1b. Next, NPC2 mediates the transfer to lysosomal NPC1a. Once inside the cell, sterols can be used in several ways. Some will be transported to various organs or tissues (e.g., the fat body) via lipophorin. Alternatively, some are used directly as a membrane structural component or stored as cholesteryl esters catalyzed by sterol O-acyltransferase (61, 65, 81); the esterification process is bidirectional. Additionally, esterified sterols can be hydrolyzed by the lipase Magro. If cellular sterol levels are in excess, then they can be expelled back into gut lumen via ATP-binding cassette transporters (122). Dysregulation of cholesterol homeostasis can affect insect growth, nutrient accumulation, and behavior patterns (25, 53, 62, 68, 70). However, in most natural situations, insects are more likely to encounter sterol deficits than surpluses.

Niemann–Pick Type C Genes

In humans, Niemann–Pick type disease is a group of inherited metabolic disorders in which lipids accumulate in harmful quantities in various tissues, including the liver and brain; mutations in *NPC1* and *NPC2* are associated with dysregulation of human cholesterol homeostasis (65, 99). These two genes are also found in insects, plus *Caenorhabditis elegans*, which suggests that both genes are evolutionarily conserved (128). In insects, *NPC1* and *NPC2* play key roles in cholesterol absorption into midgut epithelial cells and intracellular trafficking, and both bind cholesterol in vitro. However, there are important differences. NPC1 is a large polytopic transmembrane protein consisting of an amino terminal domain (NTD), a sterol-sensing domain (SSD), and a Patched domain (63, 152). In contrast, NPC2 is a group of small intralysosomal and soluble proteins (64) and serves as a lysosomal transporter that delivers cholesterol directly to the NTD domain of NPC1 (31, 66). In all animals studied to date, loss of function of either *NPC1* or *NPC2* is lethal.

NPC1 likely evolved from the resistance-nodulation-cell division (RND) superfamily of bacterial transporters (51). Most insects—the exceptions being basal hemipterans such as aphids, whiteflies, psyllids, and planthoppers—have 2 *NPC1* homologs: *NPC1a* and *NPC1b*. This likely occurred through a duplication event in a common insect ancestor (152). Insect NPC1a and NPC1b have higher sequence similarity and identity to mammal NPC1 compared to the mammal NPC1 homolog, NPC1L1 (45). In insects, *NPC1a* is tied to intracellular trafficking (as is human *NPC1*), and it is expressed ubiquitously in a range of different tissues and throughout development (63, 104). Loss of *NPC1a* function has no effect on embryogenesis, but *Drosophila* larval molting generally does not occur because cholesterol is trapped in aberrant lysosome-like organelles in different tissues, including the ring gland, where molting hormone is produced (119). The transcriptional regulation of *NPC1a* ring gland expression involves a *cis*-regulatory element (RSE) in the *NPC1* promoter and the gene *broad complex* (*br*) (144). Expressing *NPC1a* specifically in the ring gland of *NPC1a* mutants can rescue function, but male adults are sterile, likely due to a malfunction of cholesterol trafficking during spermatogenesis in the testes (141). The *NPC1a* mutation also causes cholesterol to aggregate in the brain, which affects the function of neurons and, in turn, locomotion (110). Interestingly, sterol trafficking is not totally interrupted in the mutant flies, so other redundant factors must be involved in cholesterol transportation (45, 63).

In contrast, *NPC1b* is not ubiquitously expressed in insects. Instead, it is usually restricted to the midgut tissue, mirroring that of mammal NPC1L1 expressed at the apical membrane of enterocytes (columnar epithelial cells of the insect midgut) (25, 66, 140, 152). NPC1b is responsible for dietary sterol absorption in enterocytes, especially following a pulse of cholesterol enrichment after a meal (73). Its expression is negatively related to the concentration of dietary cholesterol, likely through the regulation of hormone receptor 96 (HR96) (20). Interestingly, other lipids such as linoleic acid can reduce the uptake of dietary cholesterol through downregulation of *NPC1b*

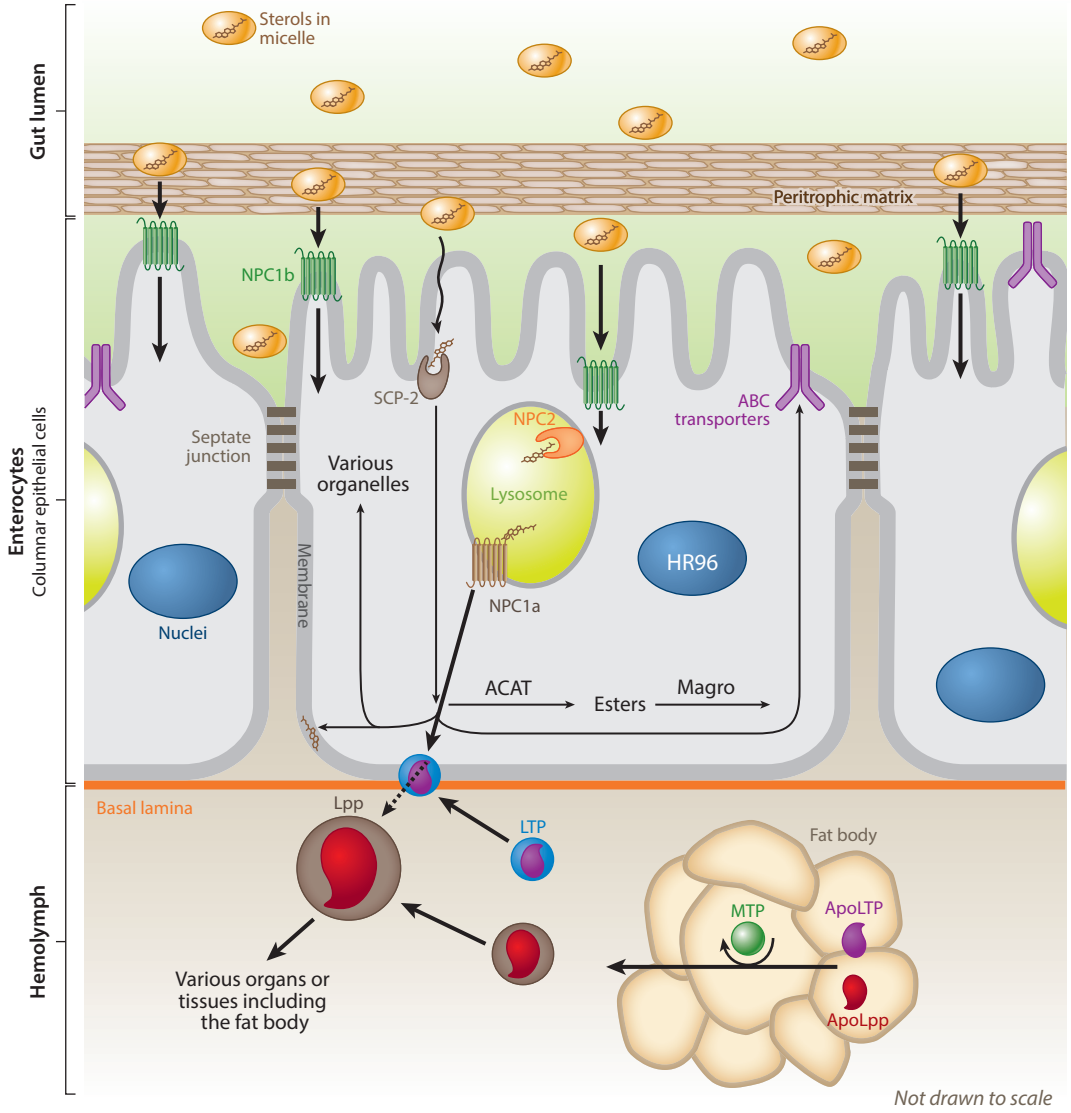


Figure 2

A graphical depiction of dietary sterol uptake, transport, and intracellular trafficking in insects. Sterol (in this case cholesterol) contained in micelles formed from ingested foods moves across the peritrophic matrix and through the enterocyte membrane (lipid bilayer) via NPC1b (the major route) (*thick line*) or diffusion (a minor route) (*wavy line*). Cholesterol entering the cell via NPC1b is transported into lysosomes, within which NPC2 mediates the transfer of cholesterol to membrane-bound NPC1a (63, 140). Cholesterol diffusing into cells is carried by SCP-2 proteins through the cytoplasm (76, 83). Once inside a cell, cholesterol has four possible destinations. First, it can be inserted into the membrane of enterocytes. Second, it can move into various organelles, including the endoplasmic reticulum and mitochondrion. Here, cholesterol can be used as a membrane structural component or form cholesteryl esters catalyzed by sterol O-acyltransferase (ACAT) (61, 65, 81). Additionally, Magro can hydrolyze sterol esters (122). Third, some cholesterol is expelled from enterocytes via ABC transporters. Fourth, and most likely, cholesterol is transported to various organs and tissues via lipophorin (Lpp). The loading of cholesterol into Lpp requires the assistance of Lipid Transfer Particle (LTP). Both Lpp and LTP, scaffolded by apoLpp and apoLTP, respectively, are synthesized in the fat body. Microsomal Triglyceride Transfer Protein (MTP) promotes the assembly of these lipoproteins (107). Finally, HR96 regulates cholesterol cellular homeostasis by coordinating cellular absorption and reverse transport, depending on dietary cholesterol levels.

(87). Moreover, the expression of *NPC1b* in *NPC1a* mutants cannot recover the wild-type phenotype. This indicates that *NPC1b* plays no role in intracellular sterol trafficking and that the two factors are not interchangeable.

NPC2 belongs to the myeloid differentiation 2-related lipid recognition protein (ML) family, and homologs have been identified in a range of organisms, including yeast, *C. elegans*, mouse, and human. Interestingly, of this group, only insects have multiple *NPC2* homologs, likely through multiple rounds of gene duplication (64, 74); insect NPC2a is the most similar homolog to human NPC2. *Drosophila* has eight NPC2 homologs, and each has the conversed disulfide bond–forming cysteine residues to form the potential hydrophobic cholesterol-binding core (46, 80). However, not all *NPC2* homologs are equally distributed across different insect tissues, and different homologs can express highly in the same tissue. However, the redundancy of NPC2 homologs in insects may be functionally significant given the nutritional requirement for sterols and the need for each cell to practice intracellular sterol transport.

Hormone Receptor 96

HR96 is a nuclear receptor consisting of a highly conserved DNA-binding domain—for regulating downstream transcription factors—and a less conserved ligand binding domain. There are 22 groups of nuclear receptor in insects, and HR96 belongs to NR1J1 (17, 41). The transcripts of HR96 are mostly confined to three tissues—the gastric caeca of the midgut, the fat body, and the Malpighian tubules—that are closely associated with nutrient processing (49, 79). The in vivo ligands for HR96 are undetermined (61), but they can bind cholesterol in vitro. They express a basal level of activity in the absence of cholesterol and are downregulated by elevated dietary cholesterol (20, 61). HR96 senses cholesterol (or its derivatives) and transduces this information to downstream factors, similar to the way Liver X receptors (LXRs) can sense sterols intracellularly in mammals (38, 44, 75). Specifically, HR96 responds to dietary sterol concentrations. For example, when flies are reared on low-sterol diets, *Drosophila* HR96 promotes genes (e.g., *NPC1b* and *NPC2*) related to cholesterol uptake and reduces the expression of those linked to cholesterol esterification (e.g., sterol O-acyltransferase) and efflux (e.g., Magro and ABC transporters) (61). Additionally, some genes (e.g., *NPC1a*) respond to cholesterol independently of HR96. HR96 is found in nearly all insects but is noticeably absent in aphids (17). Equally notable is that NPC1b is also missing in aphids (152).

Sterol Carrier Protein-2

Sterol carrier protein-2 (SCP-2)—with its conserved tertiary structures—belongs to the lipid transfer protein-2 gene family; it is a small soluble protein abundant in the cytoplasm in vertebrates and insects (39, 55, 83, 90). In vertebrates, SCP-2 is involved in the transfer of newly synthesized cholesterol from the ER to the plasma membrane (65). In contrast, insect SCP-2—which has strong binding affinity to cholesterol—is enriched in organs involved in cholesterol absorption, transportation, and metabolism. It has been suggested that SCP-2 may also help desorb and transfer dietary sterols from the enterocyte apical membrane through the cytoplasm to the basal membrane of enterocytes (in contrast to the NPC1a- and NPC1b-dependent pathway stated above) (as illustrated in **Figure 2**) (12, 76). Overexpression of SCP-2 can promote the cellular uptake of cholesterol, while its knockdown reduces dietary cholesterol absorption.

In lepidopteran insects such as *B. mori*, *M. sexta*, *Helicoverpa armigera*, *Spodoptera littoralis*, and *Spodoptera litura*, a single *SCP-x/SCP-2* gene encodes two isoforms, one for SCP-2 and the other for a fused SCP-X (a thiolase) and SCP-2 (C-terminus) protein. Some of the fused protein can then be proteolytically cleaved into SCP-X and SCP-2 (36, 50, 55, 76, 90, 129). This is similar to

SCP-2 expression in vertebrates. In contrast, the dipteran *A. aegypti SCP-x/SCP-2* gene can only proteolytically produce the cleaved SCP-2 protein, not the corresponding transcripts (40, 83, 84, 109). Additionally, *A. aegypti* also has an independent sterol-trafficking SCP-2 homolog that shares low identity (24%) with the one derived from the fused gene (16, 84).

Lipoproteins

Following uptake into midgut cells, sterols can be transferred to other organs in the insect body, but they must first pass through the aqueous hemolymph, which baths insect organs and carries resources (e.g., amino acids, sugars, lipids, ions and salts) to them. Insect lipophorin—a type of lipoprotein—resides in the aqueous hemolymph and shuttles sterols from enterocytes to various organs (24). Lipoprotein metabolism in insects and the roles of lipoproteins in the transport of fat and other lipids have been extensively reviewed elsewhere (116, 136, 143). Lipophorin loads sterols at the gut using Lipid Transfer Particle (LTP)—a high-density apoB-family lipoprotein (as is lipophorin)—and a loss of lipophorin function causes cholesterol accumulation in enterocytes (**Figure 2**) (107, 149). Both lipophorin and LTP are exclusively secreted by the fat body, and this process requires Microsomal Triglyceride Transfer Protein (MTP). In a similar manner, mammal homologs serve as the predominant transporters of cholesterol in the blood (123). In contrast to mammals, insect lipoproteins are reusable (137). Lipophorin is also responsible for the redistribution of sterols from the fat body to other tissues during the larval wandering stage. The loading of sterols from the fat body to lipophorin, unlike in mammals, is LTP independent and follows a simple aqueous diffusion pathway (72, 73).

Factors Involved in Reverse Transportation of Sterols

Transintestinal reverse transport in insects, as in mammals, removes excess or harmful sterols. To date, two groups of genes have been found that appear to regulate this process. Insects use the intestinal lipase Magro, an enzyme equivalent to its mammal homolog LipA (**Figure 2**) (122). Magro is expressed and confined in the membrane of enterocytes and can hydrolyze cellular sterol and cholesterol esters. The *Drosophila magro* mutants display excess total cholesterol and cholesterol esters, but normal free cholesterol levels. In mammals, sterols in their free form are reverse transported via ABC transporters; the same mechanism is believed to operate in insects (**Figure 2**) (15, 20, 61, 122). Similar to the preferential efflux of phytosterols over cholesterol in mammals, insects also selectively reverse transport some sterols more than others. For example, *Drosophila* larvae reverse transported more lanosterol and ergosterol compared to zymosterol, campesterol, and brassicasterol (24). Caterpillars preferentially reverse transport cholestanol over cholesterol, possibly through two ABC transporters, and, more interestingly, reverse transport was sensitive to the relative spatial arrangement of sterol atoms (69, 71). The broader functions of these ABC transporters as they relate to sterol regulation should be investigated in more detail.

STEROL PHYSIOLOGICAL ECOLOGY

Physiological ecology investigates how an organism's physiology interacts with its environment, including available food resources, its microbiome, and other living organisms that share its habitat. All insects require a source for sterols, for multiple physiological purposes, and this requirement has been shown to affect foraging behavior in the context of several different environmental factors (7). To date, the sterol physiological ecology of plant–insect interactions has been studied the most (11), but recent work has highlighted how symbiotic microbes and fungi contribute to insect–sterol interactions and that sterol requirements can drive foraging behavior in predaceous insects.

Plant–Insect Interactions

We know more about sterol use in plant-feeding insects than in any other insect group (11), in part because many of the plant-feeding insects that have been studied (especially caterpillars) are pest species that can be reared under laboratory conditions on synthetic diets. In contrast, we know very little about the comparative sterol physiology of naturally occurring insect herbivore communities. Janson et al. (67) conducted the first study of this kind using an insect herbivore community that feeds on *Solidago altissima*, a goldenrod species that has been the focus of a large number of ecological studies (114). The sterol profile of *S. altissima* contained Δ^7-sterols (spinasterol, 22-dihydrospinasterol, avenasterol and epifungistreol), and 85% of the sterol pool was in a conjugated form. More interestingly, there were major differences in the sterol composition of the six ecologically diverse insect species (a beetle, three hemipteran phloem feeders, and two dipteran gall formers) associated with *S. altissima*. For example, cholesterol was not detected in the two gall formers but was found at trace levels in the beetle (1% of the total tissue sterol profile), at intermediate levels in the hemipteran treehopper (40%), and at high levels in one of the aphids (90%). However, in the other aphid species, cholesterol levels were low (17% of the total tissue sterol profile). This variation in cholesterol profile highlights different sterol metabolic abilities among insects that share a similar host plant and, in the case of the aphids, indicates that even closely related insects may have radically different sterol metabolic capabilities. This could reflect adaptive changes in the genes involved in sterol metabolism, which has been well illustrated in two closely related flies, *D. melanogaster* and *D. pachea* (85).

These results may also reflect differences in the sterol profile of vegetative tissue and phloem. Phloem sterol profiles have now been examined in four different plant species; in contrast to vegetative tissues, cholesterol tends to be the dominant sterol in the phloem (10). This might explain the high cholesterol level in one of the aphid species and perhaps suggests that the other aphid species may be feeding on tissues other than phloem (138). An additional point about sterols in phloem is that they can exist in three forms—free, conjugated to fatty acids, or conjugated to sugars—with conjugated sterol making up at least two-thirds of the total sterol pool (10). Glycosylated sterols would be soluble in the phloem, while free sterols and fatty acid–conjugated sterols would likely be bulk transported using a carrier protein. How conjugation affects sterol use by insects has not been examined, but we suspect that conjugated sterols can be cleaved because most insect herbivores synthesize and release esterases (e.g., sterol O-acyltransferase and Magro) and glycosidases into the midgut lumen (131).

Insect symbionts can also impact insect sterol nutrition and use. For example, the Janson et al. (67) study found differences in the sterol profile of the two dipterans that produce galls on *S. altissima* (67). One of these (*Asteromyia carbonifera*) showed sterol metabolites that closely matched the sterol profile of its fungal symbiont. The other (*Eurosta solidaginis*), which does not have a fungal symbiont association, showed sterol profiles similar to the vegetative tissue of *S. altissima*. Consistent with the limited sterol metabolism in dipteran species (as discussed above), these data suggest that *A. carbonifera* eats its fungal symbiont (mycetophagy), while *E. solidaginis* eats plant vegetative tissues. Furthermore, given that cholesterol was not recovered in either species, these aphids likely do not use 20-hydroxyecdysone as their molting hormone. Additional examples of fungal symbionts aiding in sterol nutrition have recently been shown, including for grape berry moths (94), anobiid beetles (101), brown planthoppers (145), rice planthoppers (105), and two weevils (13). In contrast, Thompson et al. (132) showed that the xylem-feeding woodwasp *Sirex noctilio*, which has a symbiotic fungus, does not assimilate fungal sterols. Rather, three derivatives (e.g., cholestanol, cholestan-3-one, and cholest-4-en-3-one) were found in *S. noctilio*. These three derivatives are rarely observed in insects or plants in nature, but they do occur in tobacco plants expressing the 3-hydroxysteroid oxidase gene from an *Actinomyces* spp. bacteria (58).

S. noctilio contains a rich bacterial flora, including *Actinomyces* spp. (1). Perhaps these bacteria, which mostly reside in the gut lumen, provide metabolic enzymes that generate the atypical sterols in *S. noctilio*. On a related note, all insects are colonized by a group of bacteria (34) that are generally incapable to synthesizing sterols (26). However, we currently know very little about the role that bacterial flora (especially those in the midgut lumen) might play with respect to sterol metabolism.

Predator Foraging Behavior

Predaceous insects generally are not thought to be limited by sterols, as cholesterol is the dominant sterol in most animals (11). Recently, though, Ugine et al. (135) showed that the sevenspotted lady beetle (*Coccinella septempunctata*) grew and developed normally on an all-prey diet but suffered a complete loss of fitness (spermatogenic failure). This was a function of feeding exclusively on pea aphids, which have very low tissue sterol content. However, fitness was restored by feeding on plants or eating phytosterols or cholesterol. Thus, reproductive failure was clearly caused by a sterol deficiency in the male. With respect to male reproduction, sterols are critical for membrane remodeling when each syncytial spermatid is individually assembled into its own plasma membrane during spermatogenesis (106). In addition to a deficit of dietary sterols, defects in sterol trafficking may reduce the supply of sterols to important insect tissues. If sterol trafficking to the testes is defective, then spermatogenesis would be negatively affected (91, 141).

Lady beetles also demonstrated a state-dependent sterol-specific appetite and redressed their sterol deficit by feeding on plant foliage. The proximate forces that create omnivores out of carnivores have long puzzled ecologists, and this elegant study shows that sterols might be a key factor driving omnivory more broadly in insects. Pollen feeding in the omnivorous lady beetle *Coleomegilla maculata* also increases fecundity, and sterols have been implicated as the mechanism (112). Finally, cholesterol obtained through adult nutrition has been shown to impact fitness (viability) in a synovigenic parasitoid (95). A key implication for all of these species is that cholesterol plays an important role in spermatogenesis (91).

APPLIED IMPLICATIONS

Exploiting Sterol Constraints as a Novel Strategy to Control Insect Pests

Pesticide resistance from overuse is an important issue, as is a pesticide's lack of target specificity. The inability of insect herbivores to synthesize sterols, combined with the constraints on their ability to use particular types of sterols and steroids, can be exploited to develop new insect pest control strategies. A sterol-based approach also has the benefit of being target specific.

The disruption of biochemical pathways in the conversion of phytosterols into cholesterol in insect herbivorous pests has been heavily studied, and many inhibitors were discovered in the 1990s (124, 126). These substrates block enzymatic reactions by competing with phytosterols and subsequent metabolites, but the specific mode of action remains unknown, in large part because of a lack of genetic information. However, high-throughput screening and current molecular biology techniques are providing opportunities to identify the genes involved in sterol metabolism and screen for new compounds (19, 71, 78). For example, these methods helped identify SCP-2 inhibitors with high larvicidal activities in mosquitos and lepidopteran insects (36, 76, 78, 86, 150). These compounds disrupt the regular exogenous sterol supply for insects and have low cytotoxicity in mammals. Among these, α-mangostin—derived from the tropical fruit mangosteen—exhibits a promising future as a new organic pesticide (77, 86). NPC1b, the key factor in dietary sterol absorption, is another good target to be exploited, and the inhibitors for NPC1L1 (the homolog in mammals) are now used as cholesterol-lowering agents in humans

(152). Moreover, computer-aided exploration of proteins related to sterol metabolism can facilitate the design of novel pesticides (90, 150).

Alternatively, it might be possible to modify plant sterol and steroid profiles to control insect herbivore pests (68). Studies have suggested that it is not necessary to eliminate all phytosterols to generate genotypes that are resistant against insect pests, which is important because a minimum level of typical phytosterol is required for essential physiological functions in plants (18, 19, 70). Instead, modifying the ratio of sterols or steroids beyond a particular threshold can significantly inhibit insect population growth (3). Such an approach is environmentally friendly, with minimal effects on the nontarget species (and can work in concert with integrated pest management strategies). The major pests in any given agricultural system are usually predictable, and their dietary sterol requirement can generally be identified by comparing the insect tissue sterol profile to that of their typical host plant(s) and confirmed using artificial diets. With this information in hand, crop plants can be genetically modified to express sterols that do not meet the requirements of the pest insect.

Insects as Model Organisms to Understand Sterol Biology in Vertebrates, Including Humans

Insects and *C. elegans* share most of their basic sterol trafficking functions with vertebrates, and both insects and *C. elegans* are often used as models for studying sterol biology in animals. Additionally, compared to vertebrates, insects and *C. elegans* have several traits in their favor. These include short generation time and high fecundity, as well as the fact that they are generally less expensive to feed and maintain. Moreover, both are highly amenable to sophisticated genetic manipulations, which provide more opportunities for exploring sterol nutrition and homeostasis from a functional genomics perspective. However, in our opinion, insects have some unique advantages as a model system in studying sterol biology. First, the nutritional requirements of insects and the underlying molecular basis largely resemble those of vertebrates (22, 52). Second, *C. elegans* lacks fat-storing adipose cells; instead, they primarily store fat in epidermal cells (100). This is in contrast to insects and vertebrates, which have analogous adipose tissues (for insects, the fat body) where cholesterol and other fats are stored (107, 121, 122). Moreover, there is no good synthetic food media for *C. elegans*, in contrast to insects (52). The use of synthetic food is critical for investigating trace nutrients, like sterols, and is especially important for studying nutrient interactions, as exemplified by *Drosophila* (113). However, we recognize that every model system has its limitations, and insect models are no exception. We also recognize that candidate factors initially identified in insects will need to be verified in vertebrates, including humans.

PERSPECTIVES AND FUTURE DIRECTIONS

Great progress has been made in insect sterol biology since Hobson (59) first discovered that insects were sterol-auxotrophs. However, many questions remain. In this section, we outline areas that we feel are particularly deserving of attention. First, the genes involved in phytosterol metabolism have yet to be fully discovered. Lepidopteran larvae are good organisms for this research, but a current problem is that they are not sensitive to RNAi, which makes confirmation of gene function difficult. However, CRISPR/Cas9 allows functional genetic work on caterpillars (151), and in vivo research on insect sterol nutrition is expected in the near future. Second, sterol levels are tightly regulated in the insect body, partially through NPC1b. Moreover, flies actively expel excess sterols from their cells (122). However, the mechanisms governing these reverse transportation processes are not well understood. Third, given recent evidence that sterol

nutrition mediates interactions between bacterial symbionts, hosts, and pathogen agents, partially through the competition for sterol nutrients (23, 57, 108), more attention should be directed toward understanding the mechanisms by which sterols facilitate these interactions. Fourth, some insects (e.g., *D. melanogaster* larvae) can selectively allocate different sterols to particular tissues (e.g., cholesterol to the central nervous system). Understanding the molecular mechanism by which this occurs could lead to the development of target-specific control agents. For a range of *Drosophila* species, which are emerging pests for the berry industry and are insensitive to the dysfunction of phytosterol metabolism, such an approach has exciting potential. Addressing these collective gaps will allow us to more fully appreciate insect sterol nutrition and potentially lead to broader practical impacts.

SUMMARY POINTS

1. Insects use sterols (amphipathic molecules) in three critical ways: (*a*) as structural components in the cellular membrane, (*b*) as precursors to steroid hormones, and (*c*) as signaling molecules.
2. Cholesterol is the most common sterol reported in insects, but it occurs in very low levels in plants and is absent in fungi.
3. Insect herbivores produce cholesterol by metabolizing phytosterols, but the degree of conversion efficiency can differ between even closely related species.
4. HR96 is the master regulator of dietary sterol absorption, esterification, and efflux in insects.
5. NPC1b transfers dietary sterols into enterocytes in most insects except basal hemipterans (e.g., aphids, whiteflies, psyllids, and planthoppers).
6. NPC1a, NPC2, and SCP2 regulate intracellular sterol trafficking, while lipoproteins in the hemolymph shuttle sterols between organs (e.g., the midgut and the fat body).
7. Sterol nutrition mediates plant–insect interactions, sometimes facilitated via bacterial and fungal symbionts, and can drive predaceous insects to omnivory.
8. Insect sterol metabolic constraints can be exploited to control economically important insect pests and better understand sterol biology in mammals, including humans.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

First, we want to recognize and thank our long-term collaborator Dr. Robert J. Grebenok, a specialist in sterol biochemistry. Many of the topics and ideas that we cover in our review have been informed by discussions with him over the past three decades. We also thank Xiaoqing Zhang for reading and editing drafts of the manuscript. This work was supported by funds awarded to X.J. by the National Key R&D Program of China (2017YFD0200400), the National Natural Science Foundation of China (31572058), and the Shaanxi Natural Science Foundation (2017JM3005); it

was also supported by funds to S.T.B. from the National Institute of Food and Agriculture (NIFA) program via the United States Department of Agriculture (2016-67013-24762).

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