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REVIEW

Extracellular nutrient digestion and absorption in the insect gut

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Abstract

Insects are the most abundant and diverse class of animals on the planet. One explanation for their success is their extraordinary ability to successfully consume a wide range of foods. Like all heterotrophic organisms, insects need to acquire vital nutrients from their diet. The central organ for food digestion and absorption of nutrients is the gastrointestinal tract. This organ's principal functions are mediating the efficient digestion of the diet and protecting the organism against harmful chemicals, microorganisms, and mechanical damage from the food. These functions are achieved through regional differentiation of the alimentary canal as well as highly flexible adaptations to the consumed diets, both at anatomical and molecular levels. Numerous studies describing the general gut morphology and associated digestive mechanisms of various insects exist. Nevertheless, the molecular patterns underlying digestion and nutrient uptake in insects are still poorly characterized. This review aims to provide an overview of the general strategies of extracellular macronutrient digestion and consequent nutrient absorption found among different orders of insects.

Keywords Insect · Gastrointestinal tract · Digestive enzymes · Nutrient digestion · Nutrient absorption

Introduction

Insects are among the most successful organisms on the planet. Together, they are able to consume almost every available food source and survive in some of the most extreme circumstances. Insects are heterotrophic organisms, and thus need to acquire essential nutrients from the environment via digestion. Diverse ways of feeding, including herbivory, carnivory, and hematophagy are supported within the insect class of the animal kingdom, and each type of feeding strategy is characterized by specific structural and molecular adaptations (Chapman 2013).

In all insects, the principal site for food digestion, and subsequent absorption of vital nutrients, is the gastrointestinal tract. The insect digestive system has been a subject of study for many decades, with gut physiology and the associated digestive mechanisms found in various insect species being especially well-documented. In general, the morphology of the gastrointestinal tract is comparable in all insects (Fig. 1). After the food has entered the body through the mouth, it undergoes both mechanical and enzymatic degradation inside the intestinal lumen to obtain essential macronutrients (Fig. 2). The entire gastrointestinal tract of an insect is precisely modified at both the anatomical and molecular level to efficiently match its feeding habits and diet (Chapman 2013). Since insects exhibit divergent feeding habits, there are numerous species-specific digestive repertoires mediating the efficient enzymatic breakdown of each of their preferred diets. These specific enzymatic profiles have been described in detail for many insect species. In contrast, the molecular patterns underlying the regulation of food degradation and nutrient uptake in insects are still poorly understood and documented (Chapman 2013; Miguel-Aliaga et al. 2018).

Because of the many parallels with vertebrate metabolic pathways, insects are useful and accessible organisms for studying gut physiology. The aim of this review is to provide an overview of the general strategies of the intrinsic extracellular macronutrient digestion and consequent nutrient absorption of insects. This review will start with a general overview of the insect gastrointestinal tract morphology. Next, the general extracellular enzymatic digestion inside the intestinal

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lumen will be described. Last, an overview of the different pathways of nutrient absorption will be provided.

General anatomy of the insect gastrointestinal tract

The alimentary canal of insects runs through the entire body cavity from mouth to anus and is divided into three main regions: foregut, midgut, and hindgut (Fig. 1). The epithelia of both foregut and hindgut are of ectodermal origin and have a cuticular inner lining. The midgut epithelium is the only part of the alimentary duct that is of endodermal origin and consists of a single layer of cells, lacking a cuticular lining. Therefore, the majority of the enzymatic digestion and the absorption of nutrients largely take place in the midgut (Chapman 2013).

The insect mouth, through which food enters the body, is directly connected to the foregut. The foregut usually consists of four distinct areas: the pharynx, esophagus, crop, and proventriculus. The pharynx and esophagus are both involved in the distal movement (by peristalsis) of the food towards the crop. The food bolus is then stored in the crop, until it is passed on to the midgut for digestion. Some initial enzymatic digestion occurs in the crop, which is mediated by salivary enzymes and enzymes regurgitated from the midgut. In most insects, the crop is situated in line with the alimentary canal and expands laterally. However, in adult Lepidoptera and adult Diptera, the crop is an associated lobe of the esophagus (Chapman 2013: Stoffolano and Haselton 2013). The proventriculus is located at the transition of the foregut to the midgut. This muscular region is an important valve for the passage of food and is in some insects, mostly Orthoptera, Blattodea, and some Coleoptera, even responsible for the additional mechanical degradation of the food through the action of modified tooth-like chitinous structures. Next, the food bolus enters the midgut—the primary site of enzymatic digestion in insects. Most insects possess two to eight small pouches, called caeca, diverging from the proximal end of the midgut. These gastric caeca provide extra surface area for enzyme secretion and biochemical digestion. Hence, they strongly increase the digestive efficiency of the midgut. After enzymatic digestion in

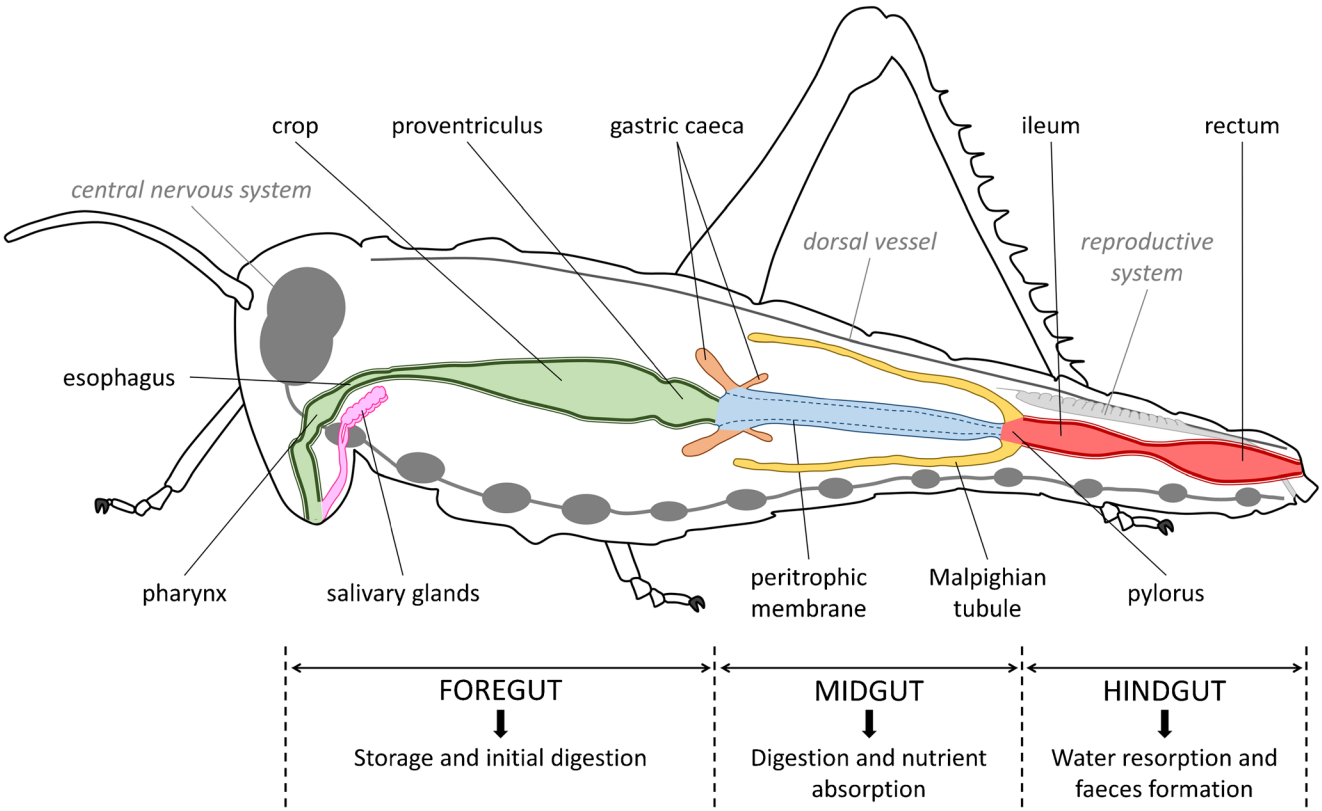

Fig. 1 General anatomy of the insect gastrointestinal tract. The insect intestine is divided into three main regions: foregut (green), midgut (blue), and hindgut (red). The foregut consists of the pharynx, esophagus, crop, and proventriculus. The midgut is divided by the peritrophic membrane into an endoperitrophic and ectoperitrophic space. The hindgut encompasses the pylorus, ileum, and rectum. Each area is specifically adapted to its respective function in digestion, with the majority of enzymatic digestion occurring inside the lumen of the midgut and associated caeca (orange). The Malpighian tubules (yellow) are connected to the pylorus of the hindgut. The salivary glands are drawn in pink. The cuticular lining of the foregut and hindgut are indicated by thicker lines

the midgut, the remaining food bolus passes on to the hindgut. The hindgut's general function is the osmotic regulation of internal fluids. The hindgut can be divided into three distinct regions: the pylorus, ileum, and rectum. Malpighian tubules are specialized tubular excretory organs that are attached to the pylorus (Maddrell and O'Donnell 1992). Inside the pylorus, the food bolus and the secretions from the Malpighian tubules are mixed. To prevent the reflux of the food bolus back into the midgut, the pylorus is surrounded by circular muscles to act as a sphincter. The ileum connects the pylorus with the rectum and can harbor important symbiotic microorganisms. For example, in termites and scarab beetles, microbiota inside the ileum contributes largely to the degradation of plant polysaccharides utilized for aerobic metabolism by the insect (Douglas 2015). The third and final part of the hindgut, the rectum, is the main site for ion and water exchange. In order to support its role in osmosis, the cuticular lining of the rectum is substantially thinner than that of the other regions of the hindgut (Chapman 2013).

Gut motility results from the presence of muscular layers in the wall of the alimentary canal. The entire alimentary canal is enclosed by striated circular and longitudinal muscles. The circular muscles directly enclose the gut and are surrounded by an external layer of longitudinal muscles. The muscles surrounding the fore- and hindgut are the most developed, since these parts of the gut function as dilators. The gastrointestinal tract of insects generally has three profoundly innervated regions: the foregut/anterior midgut, the midgut/hindgut junction, and the posterior hindgut (Hartenstein 1997; Cognigni et al. 2011). These innervations largely control the peristaltic regulation of the visceral muscles to promote the intestinal food transit, but might also regulate epithelial functions, such as enzyme production and nutrient absorption (Cognigni et al. 2011; Lemaitre and Miguel-Aliaga 2013).

Anatomy and functioning of the insect midgut

The general morphology of the midgut epithelium is comparable in all insects. It consists of a single layer of epithelial cells, containing four cell types: the intestinal stem cells (ISCs) and the enteroblasts (EBs), which are immature cells that can further differentiate into enteroendocrine cells (EECs) or into enterocyte cells (ECs) largely depending on the Notch/Delta and Wingless signaling pathways (Tanaka et al. 2007; Takashima et al. 2011; Miguel-Aliaga et al. 2018) (Fig. 2). EECs are part of the enteroendocrine system and represent a vast proportion of cells present in the midgut epithelium. These cells act as sensors for the internal intestinal

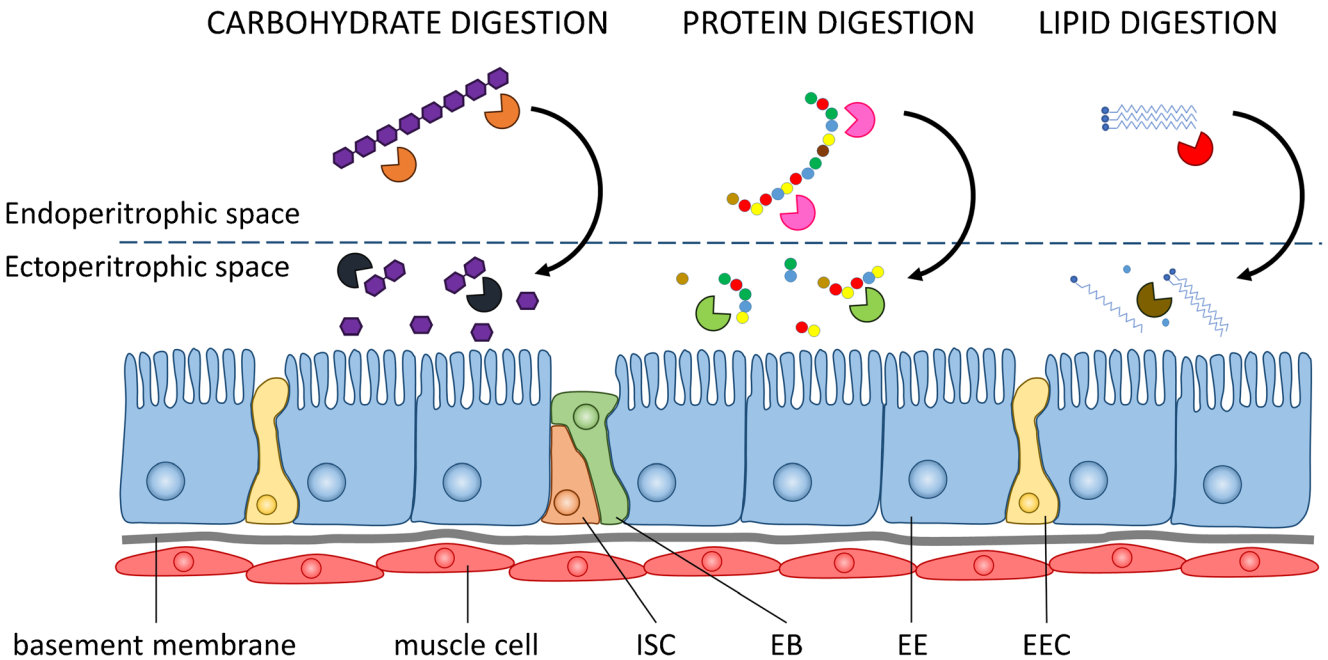

Fig. 2 Compartmentalized digestion of macronutrients inside the midgut. The midgut epithelium is a single layer of epithelial cells, containing four cell types: the intestinal stem cells (ISCs), enteroblasts (EBs), enteroendocrine cells (EECs), and enterocyte cells (ECs). Gut motility results from the presence of muscular layers in the wall of the alimentary canal. ECs bear a brush border of microvilli on the apical membrane, produce and release digestive enzymes, absorb the digested end products from the midgut lumen, and regulate the midgut luminal environment. The midgut also synthesizes the peritrophic membrane (PM, blue dashed line). The PM compartmentalizes the midgut into an endo- and ectoperitrophic space, which gives rise to the spatial organization of digestion, with initial macronutrient digestion occurring inside the endoperitrophic space, and the assimilation of digestive end products occurring inside the ectoperitrophic space. Distinct digestive enzymes are active in the separated areas of the midgut

environment and are able to produce specific gut regulatory hormones and peptides, as well as mediate the communication of the nutritional status to other organs (Sternini et al. 2008). Well-known examples of enteroendocrine peptides in insects are allatostatin A, myosuppresin, neuropeptide F, and tachykinin-related peptides. For a more in-depth summary on the presence and functions of enteroendocrine peptides in different insect species, the reader is referred to reviews by Spit et al. (2012a) and Wegener and Veenstra (2015). ECs are the most prevalent cells in the midgut epithelium. They are characterized by a columnar shape and bear microvilli on the apical membrane that significantly increase the surface area of the midgut. The enterocytes of the midgut are responsible for the production of digestive enzymes and the absorption of digested products (Huang et al. 2015). In addition, in some insects, enterocytes are also specialized in controlling the midgut luminal pH. In general, the midgut lumen of most insects has a neutral pH between 6 and 7.5. Exceptions are the more acidic to neutral midgut pH (pH 5–7) of most Coleoptera, the highly alkaline midgut pH of Lepidoptera (pH > 9), and specialized acidic anterior midgut regions of most Diptera and Hemiptera (Terra et al. 1994; Chapman 2013). In *D. melanogaster* and other higher dipterans, specialized ECs, named coprophilic or copper cells, are responsible for the acidification of the anterior midgut (Dubreuil et al. 1998). The acidic environment is generated by a membrane-associated H^+ V-ATPase pump, a carbonic anhydrase and five transporters or channels mediating K^+, Cl^-, and HCO_3^- transport (Overend et al. 2016). The acidic pH is important for the initial breakdown of food, as well as for protection against pathogens. In contrast to the well-described mechanism of acidification, less is known about how the pH in other midgut areas is maintained (Miguel-Aliaga et al. 2018). This pH-associated compartmentalization inside the *Drosophila* midgut is remarkable, but not unique in insects. In the midgut of lepidopteran insects, specialized ECs, called goblet cells, are responsible for the global alkalization of the midgut lumen. Goblet cells form cavities inside the midgut epithelium and mediate the active transport of K^+ into the gut lumen via $K^+/2H^+$ antiporters driven by a V-ATPase pump (Flower and Filshie 1976; Chapman 2013). The gut pH is one of the most important regulators of digestive enzyme activity in insects. Consequently, different enzyme profiles are found among different insect orders, with a clear correlation between the gut pH and the pH optima of the observed digestive enzymes. Maintaining the gut pH is therefore a pivotal role of the midgut epithelium (Terra and Ferreira 1994).

In most insect species, a membranous, noncellular structure is observed inside the midgut. This semipermeable membrane is the peritrophic matrix (PM) and envelops the food bolus (Fig. 3). The PM is composed of chitin microfibrils embedded in a matrix of proteins (peritrophins), glycoproteins, and proteoglycan. Two types of PMs exist according to their respective way of synthesis as reviewed by Lehane (1997). Type I PMs are produced by the entire midgut and can be found in the majority of PM producing insects. This type of PMs can be generated continuously, found in continuous feeders, such as locusts and cockroaches, or in response to feeding, found in batch feeders, such as mosquitos. Type II PMs are produced by restricted areas in the anterior midgut and are usually only found in Diptera, Dermaptera, Embiodea, and some families of Lepidoptera (Lehane 1997). In some insects, however, both types of PM can occur at different life stages. For example, in mosquitos, larvae produce a type II PM, while adult females produce a type I PM (Dinglasan et al. 2009). Not all insects produce the PM, and some orders, such as Hemiptera and Thysanoptera, have evolved an analogous extracellular lipoprotein structure called the perimicrovillar membrane (PMM) (Lehane 1997; Terra 2001; Silva et al. 2004). Generally, the PM encloses the food bolus to separate the food from the midgut epithelium, thereby protecting the midgut epithelium from mechanical and chemical damage by the food bolus, as well as from harmful pathogens and toxins (Huang et al. 2015). As a semipermeable membrane, the PM also controls the passage of molecules, hence dividing the midgut lumen into two physiologically separate regions: the endoperitrophic space (inside the PM) and the ectoperitrophic space (outside the PM). The compartmentalization of the midgut lumen results in the spatial organization of digestive events. In the endoperitrophic space, food flows from the anterior to the posterior end of the midgut. The pore size at the anterior side of the PM allows the movement of digestive enzymes into the endoperitrophic space. The initial digestion of the food thus takes place inside the endoperitrophic space, where most large macromolecules are degraded. The pore size of the PM gradually decreases towards the posterior end, only allowing smaller sized molecules to pass through the membrane to the ectoperitrophic space (Gutiérrez-Cabrera et al. 2016). Inside the ectoperitrophic space, a countercurrent flow pushes the food particles retrograde towards the caeca. This flow is created by the excretion of water and ions by the Malpighian tubules and their subsequent uptake by the caeca (Fig. 3). In addition, neuropeptides also influence gut motility as well as gut contents, thereby affecting the digestive flux (Spit et al. 2012a). For example, in the migratory locust, *Locusta migratoria*, the neuropeptide sulfakinin mediates the clearing of food contents from the caeca (Zels et al. 2015). Whenever food particles become small enough to diffuse through the PM, the countercurrent flow in the ectoperitrophic space will push them back towards the caeca where digestion continues. From the caeca, food particles can again enter the endoperitrophic space

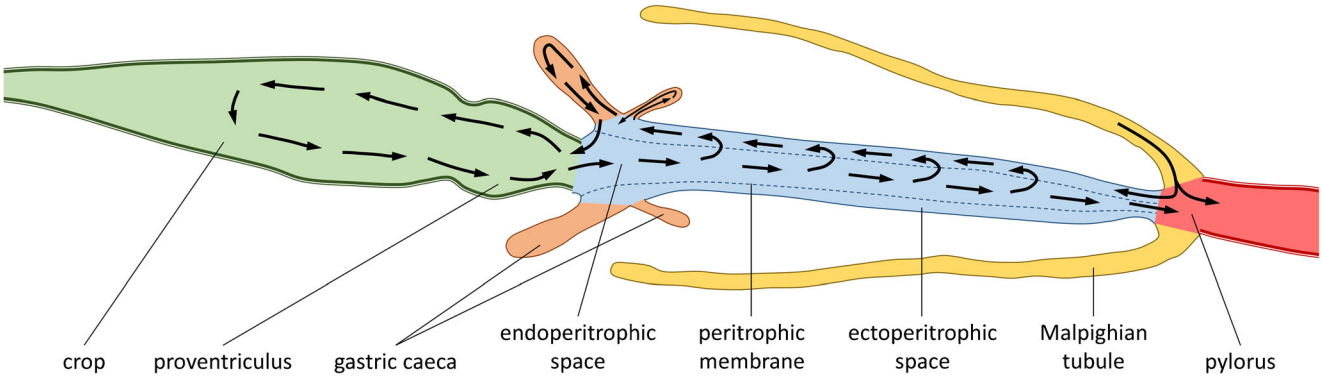

Fig. 3 Flow of food particles inside the insect gut. The countercurrent flow inside the midgut is created by the secretion of water and ions by the Malpighian tubules (yellow) and their subsequent uptake by the caeca (orange). In the endoperitrophic space, food particles flow towards the distal end of the midgut, while inside the ectoperitrophic space food particles are transported proximally towards the caeca. Some midgut contents may even re-enter the crop. The arrows indicate the path followed by food particles inside the midgut. The countercurrent flow inside the midgut strongly increases digestive efficiency

restarting the digestive cycle. The recycling of food particles and digestive enzymes strongly increases digestive efficiency inside the insect midgut (Lehane 1997; Terra 2001; Chapman 2013; Bolognesi et al. 2008). The eventual end products of digestion will be taken up from the ectoperitrophic space by the midgut epithelial cells (Chapman 2013).

Extracellular enzymatic nutrient digestion

To digest dietary proteins, carbohydrates, and lipases, insects rely on the hydrolytic action of digestive enzymes (Fig. 2; Table 1). The composition of digestive enzymes mediating the degradation of food inside the gut is complex and often species-specific. It is largely determined by feeding habits, ingested food quality and quantity, and specific midgut luminal environments. The evolutionary relationships between insects and their hosts have also stimulated this complexity in great ways. This section will provide a comprehensive overview of the enzymatic digestion and related digestive enzyme classes found in different insect orders.

Protein digestion

The adequate uptake of proteins is pivotal for the survival of any insect. Proteins are digested to release their amino acid contents, which are then absorbed across the midgut epithelium to be used in vital processes, such as growth and development, energy storage, and reproduction. Some amino acids cannot be synthesized de novo by the insect and need to be acquired from the environment. These essential amino acids for insects are the aromatic phenylalanine, tryptophan, and histidine; the aliphatic leucine, isoleucine, valine, and threonine; the sulfur-containing methionine; and the basic arginine and lysine (Boudko 2012).

Different proteolytic enzymes, also called proteases, mediate protein breakdown (Table 1). In general, digestive proteases are divided into two groups: the endopeptidases that cleave the proteins internally and the exopeptidases that cleave the terminal amino acids from the proteins. The

Table 1 General overview of macronutrient digestion in the insect's gastrointestinal tract

	Protein digestion	Carbohydrate digestion	Lipid digestion
Enzymes	Exopeptidases ○ Carboxypeptidases ○ Aminopeptidases Endopeptidases ○ Serine proteases ○ Cysteine proteases ○ Aspartic proteases ○ Metalloproteases	α-Amylases α/β-Glucosidases Endo/exo-β-1,4-Glucanases (cellulases)	Lipases Phospholipases Sterol reductases
Expression	Caeca, midgut	Salivary glands, caeca, midgut	Caeca, midgut
Substrates	Proteins, polypeptides	Polysaccharides, disaccharides	Acylglycerols, fatty acids, galactolipids, phospholipids, sterols
Products	Amino acids, oligopeptides	Mono- and disaccharides	Free fatty acids, phospholipids, cholesterol

exopeptidases are further divided into two groups: the carboxypeptidases that cleave at the carboxylic terminus and the aminopeptidases that cleave at the amino terminus of proteins. Proteases are typically classified into four groups, based on the composition of the catalytically active site: (1) the serine proteases, having a serine residue at the active site; (2) cysteine proteases, having a cysteine residue at the active site; (3) the aspartic (acid) proteases, having an aspartyl residue at the active site; and (4) the metalloproteases, having a metal ion at the active site (Rao et al. 1998; Berg et al. 2002). In general, insect digestive proteases rely on similar catalytic mechanisms as vertebrate digestive proteases.

Serine and cysteine proteases rely on the activity of a catalytic triad, respectively Ser-His-Asp and Cys-His-Asp, to degrade peptide bonds. Serine proteases include trypsins, chymotrypsins, and elastases. Trypsins preferentially cleave peptide bonds following an arginine or lysine residue; chymotrypsins preferentially cleave after a tyrosine, phenylalanine, or tryptophan residue; and elastases preferentially cleave peptide bonds following an alanine or serine residue (Terra and Ferreira 1994; Berg et al. 2002). Cysteine proteases in insects include the cathepsin B-like and cathepsin L-like proteases (Terra and Ferreira 1994, 2012; Chapman 2013). Cathepsin B-like proteases are in fact defined as important insect peptidyl dipeptidases, instead of real endopeptidases. The cathepsin L-like proteases, on the other hand, are true endopeptidases that preferably cleave peptide bonds with hydrophobic amino acid residues (Terra and Ferreira 2012). Metallo- and aspartic proteases differ from serine and cysteine proteases because they depend on an activated water molecule instead of an amino acid as a nucleophile (Berg et al. 2002; Zhu-Salzman and Zeng 2015). The water molecule is activated by a divalent metal cation or an aspartic residue respectively, present in the active site. The most prevalent metalloproteases in insects are the zinc metalloproteases, representing a major fraction of the exopeptidase activity found in insects (Terra and Ferreira 2005). The best-known insect digestive aspartic acid proteases are the cathepsin D-like proteases. These proteases show high sequence similarity with cathepsin D proteases, a major family of intracellular aspartic proteases in the lysosomes of all animals, and thus are probably derived from the same ancestral gene as the intracellular cathepsin D found in lysosomes (Padilha et al. 2009). The shift towards the use of lysosomal enzymes for digestion presumably evolved from adaptations to dietary challenges, such as the presence of plant-derived defensive compounds in the food (Pimentel et al. 2017).

An important factor determining the digestive enzyme profile and activity in insects is the midgut luminal pH. The pH optimum of serine proteases is neutral to alkaline. Hence, serine proteases are found in all studied insect orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera. A large phylogenetic analysis of trypsin and chymotrypsin sequences originating from over 60 different insect species belonging to the above-mentioned orders illustrated that trypsins and chymotrypsins are clearly divided into two phylogenetic clades and that trypsins or chymotrypsins originating from the same insect class cluster together inside their respective clade (Spit et al. 2014). This indicates that these proteases are specifically adapted to their associated midgut environment. Serine proteases make up for the majority (as much as 95%) of the total proteolytic activity in the gut of larval Lepidoptera (Srinivasan et al. 2006). Serine proteases isolated from Lepidoptera midguts show clear alkaline pH optima, probably as a consequence of adaptations to the alkaline midgut environment (Christeller et al. 1992). Most of the remaining proteolytic activity in Lepidoptera midguts is executed by cathepsin-B like cysteine proteases, and different exopeptidases (Christeller et al. 1992; Terra et al. 1994; Bown et al. 1997; Patankar et al. 2001; Breugelmans et al. 2009; Tabatabaei et al. 2011). The midguts of Orthoptera are characterized by a neutral pH, and in parallel, the majority of proteolytic activity in the midguts of this order results from serine proteases (Chapman 2013). Transcriptional and enzymatic studies in *Locusta migratoria* and *Schistocerca gregaria* (Orthoptera) revealed high serine protease activity in the midgut (Spit et al. 2012b, 2014). Three trypsins purified from *L. migratoria* showed maximum activity at pH 8, matching the neutral midgut environment of these insects (Lam et al. 2000). Serine proteases are also the most prevalent digestive proteases in other studied Orthoptera, including the grasshopper *Oedaleous asiaticus* and the cricket *Gryllus bimaculatus* (Huang et al. 2017; Woodring et al. 2017). Serine proteases also represent the majority of proteases identified in Dipteran midguts. Mosquitos largely depend on trypsin serine proteases for their proteolytic digestion (Borovsky 2003). Genome analysis of *D. melanogaster* has revealed a substantial amount of putative serine proteases present in the midgut. Other identified proteases were metalloproteases and, probably restricted to the acid midgut region, cysteine and aspartic proteases (Lemaitre and Miguel-Aliaga 2013). Cysteine and aspartic proteases are described to have slightly acidic pH optima and are therefore predominantly found in the acidic midguts of Coleoptera, and specific acidic midgut regions of Diptera, Heteroptera, and Hemiptera (Terra et al. 2012). The Colorado potato beetle, *Leptinotarsa decemlineata*, depends on the proteolytic actions of both cysteine proteases, with cathepsin L-like, B-like, and H-like activities, and aspartic proteases, with cathepsin D-like activity. In these insects, pH ranges from 5 to 7 along the midgut lumen. Hence the dominant protease activity shifts from aspartic proteases mediating initial proteolytic digestion at mildly acidic pH to cysteine and serine proteases for subsequent proteolytic digestion at neutral pH (Brunelle et al. 2004; Srp et al. 2016). Cathepsin D-like proteases in insects have low pH optima and were first discovered in the very acidic

anterior midgut region of *Musca domestica* (Diptera) (Greenberg and Paretsky 1955; Padilha et al. 2009) and were later also identified in other Diptera, Hemiptera, and several Coleoptera (Terra et al. 2012). For example, in *Dysdercus peruvianus* (Hemiptera), cathepsin D-like proteases active in the very acidic anterior midgut portion (pH ~ 3.5) are responsible for the breakdown of specific cysteine protease inhibitors present in their cotton seed diet. By degrading these plant defensive compounds, cathepsin D-like proteases both complement the general protein digestion as well as protect the digestive activity of the cathepsin L-like cysteine proteases in the posterior midgut regions (Pimentel et al. 2017). In *M. domestica*, the cathepsin D-like proteases in the acidic anterior midgut region have important anti-bacterial functions (Espinoza-Fuentes and Terra 1987).

Besides gut pH, adaptations to their specific feeding habits have also largely influenced the digestive enzyme profiles in insects. For example, the interaction between herbivorous insects and plants has massively impacted their digestive enzyme profiles. One common defensive strategy of plants is to inhibit the insect digestion via digestive enzyme inhibitors, such as protease inhibitors (PIs). Plant PIs are abundant proteins in all tissues susceptible to insect voracity, namely storage, reproductive, and vegetative tissues, and their expression can be induced upon wounding. Once present in the insect gut, these inhibitory proteins are able to block the proteolytic enzymes, thereby dramatically decreasing the digestive efficiency. To continue feeding on these plants, insects evolved adaptive strategies to overcome the detrimental effects of these plant PIs (Jongsma 1997; Zhu-Salzman and Zeng 2015). This evolutionary arms race has resulted in several PI-induced adaptations, typically characterized by changes in the midgut enzymatic composition. Different counter adaptive strategies include the overproduction of active proteases to overrule the inhibitory effects of PIs, a shift to inhibitor insensitive proteases, and the production of proteases capable of degrading the plant PIs (Vorster et al. 2015; Zhu-Salzman and Zeng 2015). Consequently, plant-insect interactions and the adaptation to plant defensive compounds have resulted in the positive selection for certain digestive enzyme profiles in insects. The PI-induced response has been extensively investigated in many pest insects. For example, the Lepidoptera *Helicoverpa armigera* and *Spodoptera exigua* both respond to the uptake of PIs by producing both more and inhibitor-insensitive proteases (Srinivasan et al. 2006). In the locust *S. gregaria*, in addition to induced overall proteolytic activity, increased carboxypeptidase activity was observed when the serine protease activity was inhibited by plant and/or insect-derived PIs (Spit et al. 2012b). In *L. migratoria*, a more generalized upregulation of serine protease activity in response to dietary plant serine PIs was observed (Spit et al. 2014, 2016). When fed with PI-containing diet, *T. castaneum* responded by shifting from a cysteine to a serine protease-based digestion (Oppert et al. 2005). The Colorado potato beetle appears to downregulate the expression of cysteine and aspartic proteases targeted by ingested plant PIs, while simultaneously upregulating distinct PI insensitive cysteine proteases and serine proteases (Petek et al. 2012). And as described earlier, *D. peruvianus* (Hemiptera) uses cathepsin D-like proteases in its acidic anterior midgut portion to degrade of specific cysteine protease inhibitors present in their diet. More examples of PI-induced counter defenses are described in several in-depth reviews (see for example Jongsma 1997; Mello and Silva-Filho 2002; Lopes et al. 2004; Srinivasan et al. 2006; Zhu-Salzman and Zeng 2015).

Endogenous mechanisms regulating the protease activity in insects remain largely elusive. Specific endogenous pancreatic secretory trypsin inhibitor-like (PSTI-like) proteins, according to their analogy with mammalian PSTIs, have been identified in several insect species, including *D. melanogaster*, *L. migratoria*, and *Bombyx mori.* These endogenous trypsin inhibitors probably protect the organism from prematurely activated digestive enzymes, as was firstly discovered in *L. migratoria* in 2011 by Van Hoef and colleagues (Van Hoef et al. 2011). This protective mechanism suggests that insects are able to autonomously control protease activity inside the intestine, analogous to vertebrates. Recently, a study in *H. armigera* confirmed the presence of endogenous PIs mediating the gut proteolytic activity in response to food availability (Lomate et al. 2018). Nevertheless, further research is needed to characterize these regulatory mechanisms in insects.

Carbohydrate digestion

Carbohydrates are used as a direct source of energy, or they can be either converted to lipids for energy storage or recycled as the carbon skeleton for the synthesis of various amino acids. Moreover, the insect cuticle largely consists of the polysaccharide chitin, which can be generated from simple sugars absorbed from the diet. The majority of carbohydrate digestion in insects is concentrated in the midgut, but initial digestion can already take place in the foregut and mouth. The most common sources of dietary sugars for insects are starch, cellulose, and sucrose. Carbohydrate digestion is typically mediated by digestive enzymes, carbohydrases, that break down the glycosylic bonds of polysaccharides. The end products of carbohydrate digestion are mainly monosaccharides that are absorbed across the midgut epithelium (Table 1) (Chapman 2013; Miguel-Aliaga et al. 2018). These end products in turn regulate the carbohydrate digestion rate inside the gut. For example, in *D. melanogaster*, the accumulation of sucrose, glucose, and fructose stimulates a feedback loop that inhibits the gene expression of carbohydrases and lipases (Chng et al. 2014; Miguel-Aliaga et al. 2018).

The initial digestion of carbohydrates is mediated by amylases. These types of carbohydrases catalyze the breakdown of

the α-1,4-glucan chains present inside dietary polysaccharides, such as starch and glycogen. The only type of amylases recognized in insects is α-amylases (Terra and Ferreira 1994). Alpha-amylases can act on both internal (endo-amylases) and external (exo-amylases) linkages in a random manner, thereby producing smaller polysaccharides and disaccharides that will be further degraded to monosaccharides during subsequent steps of carbohydrate digestion performed by distinct enzymes. (Terra et al. 1994; Chapman 2013; Da Lage 2018). A recent review by Da Lage shows that most insects possess multiple gene copies encoding α-amylase digestive enzymes, called the *Amy* genes (Da Lage 2018). The copy numbers vary from one to as much as 12 *Amy* genes and can vary drastically within the same insect genus. A well-studied genus in this context is *Drosophila*, where the *Amy* gene has undergone multiple duplication events. In this insect lineage, *Amy* copy numbers vary from one in *D. virilism* to six or seven in *D. ananassae*, even though these insects grossly share the same diet (Da Lage et al. 2000; Da Lage 2018). Also in other insect orders, this drastic divergence between *Amy* copy numbers was observed. The presence of multiple *Amy* gene copies might be part of specific counter defenses against plant anti-digestive factors, such as amylase inhibitors (Franco et al. 2002). The vast majority of amylase production and activity is situated in the midgut. However, part of the carbohydrase activity can already be initiated in the mouth and foregut. This can be largely ascribed to amylases originating from the midgut that migrate towards the foregut upon food uptake, as observed in the larvae of Coleoptera, Diptera, Lepidoptera, and Orthoptera (Da Lage 2018). Interestingly, in some insects, including *H. armigera* and the mosquito *Aedes aegypti,* the salivary glands are also capable of producing some amylases themselves (Grossman et al. 1997; Kotkar et al. 2012; Da Lage 2018).

After the initial breakdown of large oligosaccharides by amylases, glucosidases mediate the subsequent degradation of oligo- and disaccharides into monosaccharides. Two types of glucosidases are found in insects: α- and β-glucosidases. The nomenclature correlates with the type of linkage the enzymes targets, namely α-1,4-glucose terminal linkages and β-1,4-glucose terminal linkages (Terra and Ferreira 1994). Glucosidase activity generates monosaccharides, the end products of carbohydrate digestion, to be absorbed by the midgut enterocytes. The substrate preference of glucosidases is largely dependent on the glucosidase size and its active site preference. The presence of different types of glucosidases in the midgut helps to regulate the efficient stepwise digestion of both long and short dietary carbohydrate chains. Glucosidases are typically highly expressed by the midgut epithelium, and dominant activity is observed in the ectoperitrophic space, as opposed to amylases, which act mainly in the endoperitrophic space (Terra and Ferreira 1994; Guzik et al. 2015). In some insects, glucosidase expression is also observed in the salivary glands (Terra and Ferreira 1994; Juhn et al. 2011). The most abundant α-glucosidases in insects are maltases and sucrases, named after their preferred disaccharide substrates maltose and sucrose (Chapman 2013). Starch and sucrose represent the dominant carbohydrate reserves in plants and are the principal sources of dietary carbohydrates for herbivorous insects. Starch is a long polysaccharide composed of a large number of glucose units and is successfully degraded inside the insect gut by the subsequent actions of α-amylases and maltases (α-glucosidase). Sucrose is a disaccharide composed of glucose and a fructose unit, and its degradation is generally mediated by α-glucosidase and β-fructosidase activity (Terra and Ferreira 1994). Recently, membrane-bound sucrose-specific sucrases (SUHs) were for the first time detected in the high-alkaline intestinal lumen of Lepidoptera larvae (Li et al. 2017). These SUHs presumably diverged from α-glucosidases to enhance the sucrose digestion, the major energy source for Lepidoptera. For Hemiptera, sap-feeding insects, sucrose is one of the most concentrated nutrients available in their diet, and accordingly, high-sucrase activity has been observed in these insects (Douglas 2006). The β-glucosidases are named after the terminal sugar unit they cleave, namely glucose. Other examples are β-galactosidase and β-fructosidase, which cleave the terminal galactose and fructose respectively. Insect β-glucosidases are part of the cellulytic system responsible for the degradation of cellulose. Interestingly, many of these enzymes have important industrial applications because of their high catalytic activity for degrading lactose (Husain 2010).

Cellulose and hemicellulose are two of the most abundant compounds in plant cell walls. These β-1,4-glucose polymers both represent a significant source of carbohydrates for plant-eating insects as their breakdown releases high amounts of free glucose molecules. Cellulose can only be completely degraded by the combined action of three sets of enzymes, often referred to as the cellulytic system, composed of endo-β-1,4-glucanases, exo-β-1,4-glucanases, and β-glucosidases. The endo- and exo-β-1,4-glucanases, sometimes referred to as cellulases, randomly target inner and outer bonds of the cellulose chain (Watanabe and Tokuda 2010). Various cellulases might be active inside the gut, such as cellulases of microbial endosymbionts, exogenous cellulases ingested via the food and endogenous cellulases. It was long believed that animals were not capable of endogenously producing cellulases, but this has been re-evaluated ever since more genomic and enzymatic evidence of cellulase activity in insects and other animals appeared (Chapman 2013). The first record of endogenous cellulase activity observed in insects was in the subterranean termite *Reticulitermes speratus* (Yokoe 1964). Since then, cellulase genes or their homologs were identified in various insect species (Watanabe and Tokuda 2010). More recently, endogenous cellulase activity has also been detected in the midguts of the well-studied insect models *T. castaneum*, the

western corn rootworm, *Diabrotica virgifera virgifera*, and *M. domestica* (Willis et al. 2011; Valencia et al. 2013; Zhang et al. 2017a, b). Remarkably, endogenous cellulases appear to be absent in *Anopheles gambiae*, *B. mori*, and *D. melanogaster* (Kunieda et al. 2006; Watanabe and Tokuda 2010). In-depth studies on the identity of these endogenous cellulases in insect midguts demonstrated that important cellulase activity was performed by the endogenously produced glycoside hydrolase (GH) family of endoglucanases. Members of the GH9 endoglucanases family have been identified in all insect orders with reported cellulase activity. Additionally, members of the GH5 and GH49 families appear to be exclusively active in Coleoptera. Although these GH cellulase families differ in structure, they do share the same substrate specificities and are suggested to have evolved convergently (Watanabe and Tokuda 2010; Kirsch et al. 2012; Chapman 2013).

Lipid digestion

All insects use lipids for energy storage in the fat body, which is accessed during subsequent periods of high energy demand or starvation (Horst 2003; Chapman 2013). Lipids are also incorporated in the growing oocyte to support oogenesis (Fruttero et al. 2017). Additionally, many insect pheromones present in the insect cuticle are synthesized from dietary lipids (Yew and Chung 2015). However, in the majority of insects, these dietary lipids seem to be less essential than proteins and carbohydrates, since insects are capable of endogenously synthesizing many fatty acids and phospholipids from dietary carbohydrates. Nevertheless, all insects do require dietary sources of sterol and polyunsaturated fatty acids (PUFAs) as structural components of the cell membrane, secondary metabolites, and starting material for steroid synthesis (Chapman 2013; Zibaee et al. 2014).

Acylglycerols, fatty acids, galactolipids, phospholipids, and sterols are the most prevalent dietary lipids consumed by insects. The key enzymes involved in lipid digestion are glycerol ester hydrolases, called lipases and phospholipases, the latter being a special family of lipases depending on a different catalytic mechanism that specifically degrade phospholipids (Turunen 1979). Lipases degrade dietary lipids to generate typical end products, such as free fatty acids, glycerols, partial acylglycerols, and phospholipid derivatives, in a process called lipolysis (Table 1) (Turunen 1979; Berg et al. 2012, Chapman 2013). In most insects, the majority of lipase production and activity takes place in the midgut and associated caeca (Majerowicz and Gondim 2013). In some insects, including *S. gregaria* and the grain aphid *Sitobion avenae*, lipases were detected in the transcriptome of salivary glands. However, they are most probably involved in insect-host interactions and their possible role in the digestive breakdown of dietary lipids remains to be elucidated (Valenzuela et al. 2003; Shukle et al. 2009; Schafer et al. 2011; Zhang et al. 2017a, b). Similar to other organisms, the insect's midgut produces many different families of lipases, namely neutral lipases, acid lipases, lipase2, lipase3, GDSL-like lipase, hormone-sensitive lipases, and galactolipases (Turunen 1979; Horne et al. 2009; Christeller et al. 2011; Chapman 2013; Gondim et al. 2018). To degrade lipid substrates, all lipases apply a similar reaction mechanism, which is typical to the α/β hydrolase fold superfamily of proteins they belong to. This mechanism is based on the active site's catalytic triad of residues, usually being Ser-His-Asp/Glu. Most lipases are capable of hydrolyzing a wide range of substrates, albeit with variable specificity. They typically cleave fatty acid residues from triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), and phospholipids. Moreover, many of these lipases can also hydrolyze carboxyl ester and thioester substrates (Terra and Ferreira 1994; Chapman 2013).

Total gut lipase content and composition varies among insect species and largely depends on the gut environment (i.e., gut pH), diet composition, and dietary requirements. Due to divergent feeding habits, insects encounter different lipid compositions in their respective diets and therefore specific repertoires of lipases with different substrate preferences are required for efficient dietary lipid uptake (Chapman 2013). For example, in the midgut of larval *Epiphyas postvittana* (Lepidoptera), six neutral and three acid lipases were detected. All identified lipases had alkaline pH optima matching the pH of the lepidopteran midgut. Sequence analyses revealed that the neutral lipases were only capable of hydrolyzing phospholipids and galactolipids, but not TAG. This correlates very well with the diet composition of the larvae, since phospholipids and galactolipids are part of the chloroplast thylakoid membranes in the green leaves consumed by the larvae. These neutral lipases probably perform the majority of lipase activity in the Lepidopteran midgut, while degradation of TAGs might be an exclusive task of the acid lipases (Christeller et al. 2011). A comparative and functional genomics screen for lipases in four main representatives of holometabolous insect species, namely *D. melanogaster* and *A. gambiae* (Diptera), *Apis mellifera* (Hymenoptera), *B. mori* (Lepidoptera), and *T. castaneum* (Coleoptera), indicated that divergent mixtures of lipases are active inside the midguts of these animals. Low lipase activity was found in *A. mellifera* and might be the result of their specialized low-fat diet. In contrast, in *T. castaneum*, a fourfold higher lipase concentration was observed, probably related to their high-lipid-containing diet (Horne et al. 2009).

Since insects are unable to biosynthesize sterols de novo, they need to acquire certain sterols from the environment, either from their diet or from gut microbial symbionts (Svoboda 1999; Chapman 2013). The dominant sterol in insects is cholesterol. Depending on their diet, insects will either directly or indirectly obtain cholesterol from their food.

Carnivorous insects obtain cholesterol directly from their food, while herbivorous and fungivorous insects synthesize most cholesterol from dietary phytosterols, mainly sitosterol and campesterol, via intermediary dealkylation pathways in their gut (Ciufo et al. 2011; Jing et al. 2012). Degradation of dietary sterols is most probably mediated inside the midgut lumen by the combined action of dehydrogenases, epoxidases, and reductases (Svoboda 1999). This has been demonstrated in several insect species including *A. aegypti*, *A. mellifera*, and *B. mori* (Svoboda 1999; Ciufo et al. 2011). Next, the resulting cholesterols are absorbed from the midgut by specific transporter systems. After absorption from the midgut lumen, cholesterol is typically incorporated into (peripheral) cell membranes or used for the synthesis of ecdysteroids, which are crucial hormonal regulators of insect post-embryonic development (Niwa and Niwa 2014).

Intestinal nutrient absorption

Once the food has been processed inside the intestine, the end products of digestion are absorbed by the apical cell membrane of the midgut epithelial cells. Absorbed molecules can then be further processed inside the epithelial cells or be released from the basal membrane into the hemolymph to be transported to their respective site of action or specific storage tissues. In this section, we will describe the most common ways of intestinal nutrient absorption in insects.

Protein absorption

The common end products of protein digestion in the insect midgut are a mixture of small peptides and free amino acids. The lipid bilayer of the midgut epithelial membrane only allows passive diffusion of a negligible amount of end products, while the vast majority need to be absorbed from the midgut lumen by both active and passive transporters located in the apical membranes of the enterocytes (Chakrabarti and Deamer 1994). Some transporter activity has also been detected in the proventriculus and the hindgut, associated with the action of the Malpighian tubules (Chapman 2013). Several amino acid transporters, with clear homology to mammalian transporter systems, have been identified in insects. These transporters include cationic acid transporters, ion-dependent and independent amino acid transporters, and oligopeptide transporters (Miguel-Aliaga et al. 2018). All identified transporters putatively mediating the trafficking of amino acids and oligopeptides across the gut epithelial membrane belong to the solute carrier (SLC) transporter family (Table 2). This SLC family consists of different types of electrochemical-energy-coupled "secondary" membrane transporters originally enlisted by the Human Genome Organization (HUGO) (Hediger et al. 2004). Generalized functional descriptions of characterized human SLC superfamily members can be found in several mini-reviews (Hediger et al. 2004; Schlessinger et al. 2013).

In insects, the majority of identified nutrient amino acid transporters (NATs) belong to the SLC6 subfamily. Within the SLC6 subfamily, insect NATs are referred to as insect NATs or iNATs (Boudko 2012). Similar to their vertebrate variants, these iNAT-SLC6 transporters are integral parts of the plasma membrane and typically use Na^+ ions to actively translocate amino acids against their concentration gradient into the cell (Bröer 2006). Multiple iNAT-SLC6 members have been identified in the genomes of various insects, but to date, only a limited number of transporters have been functionally characterized across just a few insect species (Boudko 2012; Meleshkevitch et al. 2013). To the best of our knowledge, a total of nine iNAT-SLC6 transporters have been functionally characterized in insects: two in *B. mori,* one in *L. decemlineata*, two in *A. aegypti,* three in *A. gambiae,* and one in *D. melanogaster* (Table 3). However, additional putative iNAT-SLC6 transporters were identified in the genomes of the Dipteran insects *A. gambiae* (seven members), *A. aegypti* (six members), and *D. melanogaster* (nine members), suggesting that these insects rely on expanded iNAT-SLC6 transporter networks for nutrient amino acid uptake (Okech et al. 2008). Interestingly, the few characterized iNAT-SLC6 transporters either exhibit a broad substrate spectrum, allowing the trafficking of various amino acids with a uniform affinity, or are substrate specific, only allowing the trafficking of specific types of amino acids (Boudko 2012).

Table 2 General overview of putative insect nutrient transporter systems

Amino acid absorption	Carbohydrate absorption	Lipid absorption
Amino acid ○ Insect nutrient amino acid transporters of the solute carrier family 6 (iNAT-SLC6; Table 3) ○ Solute carrier family 7 (SLC7; Table 3)	Monosaccharide ○ Glucose facilitator 2 (GLUT2) ○ Glucose facilitator 5 (GLUT5) ○ Sodium-driven glucose symporter (SGLT)	Free fatty acids ○ Fatty acid binding protein (FABP) ○ Fatty acid transport protein (FATP) ○ Scavenger receptors class B type I (Sr-BI)
Oligopeptide ○ Solute carrier family 15 (SLC15; Table 3)	Disaccharide ○ Sucrose transporter protein (SCRT)* ○ Trehalose transporter 1 (Tret1)	Sterol ○ Niemann-Pick C1 (NPC1) ○ Sterol carrier protein (SCP)

*Only characterized in *D. melanogaster* (*Dm*SCRT) (Meyer et al. 2011)

Table 3 Functionally characterized transporters of amino acids or oligopeptides

Gene name	Transporter family	Insect species	Reference
MsKAAT1	iNAT-SLC6	*M. sexta*	Castagna et al. 1998
MsCAATCH1	iNAT-SLC6	*M. sexta*	Feldman et al. 2000
DmNAT1	iNAT-SLC6	*D. melanogaster*	Miller et al. 2008
AeNAT1	iNAT-SLC6	*A. aegypti*	Boudko et al. 2005
AeNAT5	iNAT-SLC6	*A. aegypti*	Meleshkevitch et al. 2013
AgNAT5	iNAT-SLC6	*A. gambiae*	Meleshkevitch et al. 2013; Boudko 2012
AgNAT6	iNAT-SLC6	*A. gambiae*	Okech et al. 2008
AgNAT8	iNAT-SLC6	*A. gambiae*	Okech et al. 2008
LdNAT1	iNAT-SLC6	*L. decemlineata*	Fu et al. 2015
AaCAT1	SLC7-CAT	*A. aegypti*	Hansen et al. 2011
AaCAT2	SLC7-CAT	*A. aegypti*	Hansen et al. 2011
Dmslif	SLC7-CAT	*D. melanogaster*	Colombani et al. 2003
DmMnd	SLC7-HAT	*D. melanogaster*	Martin et al. 2000
DmjhI-21	SLC7-HAT	*D. melanogaster*	Reynolds et al. 2009
DmOPT1	SLC15	*D. melanogaster*	Roman et al. 1998

KAAT, K^+-coupled amino acid transporter; *CAATCH*, cation-anion-activated amino acid transporter/channel; *NAT*, nutrient amino acid transporter; *CAT*, cationic amino acid transporter; *slif*, slimfast; *mnd*, minidiscs; *OPT*, oligopeptide transporter; *iNAT*, insect nutrient amino acid transporter; *SLC*, solute carrier; *HAT*, heterodimeric amino acid transporters; *Ms*, *Manduca sexta*; *Dm*, *Drosophila melanogaster*; *Ae*, *Aedes aegypti*; *Ag*, *Anopheles gambiae*; *Ld*, *Leptinotarsa decemlineata*

Therefore, the observed iNAT-SLC6 gene enrichment and functional differentiation are believed to significantly increase the nutrient amino acid absorption spectrum and efficiency of the midgut epithelium (Boudko 2012; Chapman 2013).

The first characterized iNAT-SLC6 transporters were a K^+-coupled amino acid transporter (KAAT1) and a cation-anion-activated amino acid transporter/channel (CAATCH1) both identified in the midgut of the lepidopteran species, *Manduca sexta* (Castagna et al. 1998; Feldman et al. 2000). Remarkably, while transporters belonging to the NAT-SLC6 family are described to couple to Na^+ ions, those identified in *M. sexta* strongly couple to K^+, and less to Na^+ ions. This is an adaptation to the alkaline luminal midgut environment created by the action of V-type ATPase H^+ pumps located in the plasma membrane of the goblet cells, which retract H^+ from the gut lumen in exchange for K^+. The resulting net K^+ electrochemical gradient is used by the epithelial *M. sexta* transporters to drive amino acid absorption into the cells. Both transporters exhibit broad and overlapping substrate specificity. Additionally, the pH optima of both *Ms*KAAT1 and *Ms*CAATCH1 transporter systems also strongly correlate to the high alkaline midgut pH of Lepidoptera (Castagna et al. 1998; Feldman et al. 2000; Castagna et al. 2009). The transporters *Ae*NAT1 and *Dm*NAT1 were the first Dipteran iNAT-SLC6 transporters characterized in *A. aegypti* and *D. melanogaster* respectively and both represent close phylogenetic relatives of *Ms*KAAT1 and *Ms*CAATCH1 (Boudko et al. 2005; Miller et al. 2008a). They exhibit broad substrate specificity, but, unlike *Ms*KAAT1 and *Ms*CAATCH1, their activity depends on a Na^+ electrochemical gradient. *Dm*Nat1 also shows an unusual ability to transport D-isomers of several amino acids. This is a clear adaptation to its diet, since D-amino acids are abundantly present inside the cell walls of bacteria, which represent a considerable part of the *Drosophila* diet (Miller et al. 2008; Genchi 2017). Recent research has indicated that the Lepidopteran NATs *Ms*KAAT1 and *Ms*CAATCH1 are also capable of transporting D-amino acids across the membrane, and that their isomeric preference probably depends on the coupled cation (Vollero et al. 2016). Other characterized substrate specific iNAT-SLC6 transporters are *Ag*NAT6 and *Ag*NAT8, identified in *A. gambiae*, exhibiting high selectivity for indole- and phenyl-branched aromatic amino acids respectively (Okech et al. 2008), and *Ae*NAT5 and *Ag*NAT5, identified in *A. aegypti* and *A. gambiae*, both exhibiting high methionine selectivity (Boudko 2012; Meleshkevitch et al. 2013). More recently, the first coleopteran putative iNAT-SLC6 gene (*Ld*NAT1), exhibiting broad substrate specificity, was identified and characterized in *L. decemlineata* (Fu et al. 2015a).

The iNAT-SLC6 transporter family is clearly essential for the dietary uptake of essential amino acids. In addition, other types of transporters associated with the midgut epithelium might also contribute to the uptake of dietary amino acids and oligopeptides. Suggested candidate transporters are

members of the SLC7 and SLC15 family (Table 2). The SLC7 transporter family is well-characterized in mammals and includes the cationic amino acid transporters (CATs), mediating transport of essential cationic amino acids, and the heterodimeric amino acid transporters (HATs), transporting mainly essential amino acids across the plasma membrane (Verrey et al. 2004). Insect SLC7 transporters mediating amino acid transport into the fat body cells have been identified in *D. melanogaster* and *A. aegypti* (Martin et al. 2000; Colombani et al. 2003; Reynolds et al. 2009; Hansen et al. 2011; Boudko 2012). Nevertheless, hitherto, their contribution to amino acid uptake across the midgut epithelium membrane has not yet been experimentally confirmed. For example, the CAT-SLC7 transporter, *Aa*CAT1, characterized in *A. aegypti* is highly expressed in the fat body and shows Na^+-independent cationic amino acid transporter activity with high L-histidine selectivity at neutral pH (Hansen et al. 2011). However, the contribution of *Aa*CAT1 to the uptake of dietary amino acids can only be hypothesized, since its distribution across the alimentary tract of *A. aegypti* has not yet been investigated (Boudko 2012).

Alongside dietary amino acid uptake, the trafficking of peptides across the midgut epithelium by the action of oligopeptide transporters is also possible. This is based on the predicted parallel to mammals, where the SLC15 transporters have been identified as a family of oligopeptide transporters (Hediger et al. 2004; Schlessinger et al. 2013). However, up to date, the only characterized insect SLC15 transporter with activity in the alimentary canal is the *D. melanogaster* oligopeptide transporter 1 (*Dm*OPT1). The OPT1 transporter exhibits broad substrate specificity, with the highest selectivity for L-alanine and alanine derived short peptides, and is predicted to be involved in the initial absorption of oligopeptides in a proton-mediated manner (Roman et al. 1998). Genomic studies have revealed the presence of the SLC15 in other investigated insect genomes, including the Diptera *A. gambiae* and *A. aegypti*, indicating a putative universal function of SLC15 transporters as dietary oligopeptide transporters in insects. Though, further functional characterizations are needed to fully elucidate their functions, it is believed that both SLC7 as SLC15 transporters could potentially complement the nutrient transporter activity of iNAT-SLC6 to a certain degree. But until further investigation, their contribution to dietary nutrient uptake across the midgut epithelium should not be overestimated (Boudko 2012; Fu et al. 2015).

Carbohydrate absorption

The digestive end products of carbohydrates are mainly monosaccharides and disaccharides. These simple sugars are absorbed across the midgut epithelium into the ECs for further processing or allocation to peripheral tissues. Carbohydrates can be used as direct energy sources, but can also be stored as energy reserves, predominantly in the form of TAGs, in the fat body. In mammals, the internalization of dietary monosaccharides in intestinal cells is almost exclusively transporter mediated. Similarly, in insects, two major types of glucose transporter systems have been identified, namely the major facilitator superfamily (MFS) glucose facilitators (GLUT2 and GLUT5) and the sodium-driven glucose symporters (SGLTs). Both transporter systems belong to the SLC transporter family (Table 2) (Hediger et al. 2004). GLUTs belong to the SLC2 family and SGLTs to the SLC5 family (Scheepers et al. 2004; Kellett and Brot-Laroche 2005). Few reports on the presence and activity of GLUT and SLGT transporters in the insect alimentary tract exist (see for example Escher and Rasmuson-Lestander 2004; Caccia et al. 2005, 2007, Price et al. 2007, 2010; Bifano et al. 2010; Price and Gatehouse 2014; Kikuta et al. 2015; Govindaraj et al. 2016). However, a model for dietary sugar absorption in insects is still lacking, and therefore, most of our understanding about dietary carbohydrate absorption in insects is based on expected parallels with the classical mammalian model (Chapman 2013). In mammals, the SGLT1 and GLUT5 transporters are present on the apical membrane of the intestinal ECs, while GLUT2 is detected on both the apical and basolateral sides. Functional studies show that SGLT1 is responsible for the Na^+-mediated uptake of dietary glucose across the apical membrane of the gut lumen, GLUT5 is responsible for the facilitated transport of dietary fructose, and GLUT2 can transfer both glucose and fructose. Moreover, GLUT2 is located at both the apical and basolateral membrane and is involved in the movement of glucose and fructose both in and out the gut epithelial cells. Since homologous transporters have been identified in several insect species (see references above), similar dietary sugar uptake mechanisms are expected to be active in insects. This was for the first time experimentally observed in the hymenopteran parasitoid of aphids, *Aphidius ervi* (Caccia et al. 2005, 2007). However, to date, further experimental data supporting this mechanism of dietary monosaccharide absorption in insects remain scarce (Miguel-Aliaga et al. 2018).

A putative mechanism for the uptake of disaccharides was first discovered in *D. melanogaster*. Meyer and colleagues discovered that a protein, named sucrose transporter protein (SCRT), highly similar to members of the SLC45 transporter family, demonstrated sucrose transporter activity on the apical side of the hindgut and the vesicular membranes of ovarian follicle cells (Table 2) (Meyer et al. 2011). The apical position along the alimentary tract suggests a role in the uptake of dietary sucrose. However, until today, no other records on putative sucrose absorbing mechanisms in insects exist. Another suggested dietary disaccharide transporter is the facilitated trehalose transporter 1 (Tret1) identified in the gut of various insect species (Table 2) (Kikawada et al. 2007; Kanamori et al. 2010). Nevertheless, up to date, the

transporter function of Tret1 has mainly been analyzed in the fat body and other peripheral tissues, while its putative function in the absorption of dietary disaccharides from the intestinal lumen has largely been neglected and remains to be further investigated (Miguel-Aliaga et al. 2018).

Lipid absorption

The typical end products of lipid digestion in the gastrointestinal tract are free fatty acids, glycerol, phospholipids, and non-esterified cholesterol (Majerowicz and Gondim 2013). The majority of these compounds, together with sterol, can diffuse rather easily across the plasma membrane of the midgut epithelium, whether or not facilitated by emulsification. This emulsification is achieved by the formation of fatty acid, amino acid, and glycolipid complexes, as well as fatty acids and lysophospholipid micelles (Chapman 2013; Miguel-Aliaga et al. 2018). The majority of absorbed dietary lipids are stored as triacylglycerol energy reserves in the fat body or transported to the oocytes during female oogenesis (Majerowicz and Gondim 2013; Chapman 2013; Fruttero et al. 2017).

The diffusion of hydrophobic ligands across the plasma membranes of different tissues is facilitated by fatty acid–binding proteins (FABP). These membrane-associated cytosolic proteins have clear lipid binding potencies and were first characterized in mammals. FABPs have also been identified in several insect tissues, but a limited body of research exists on their putative nutritional role in insects (Esteves and Ehrlich 2006). Some reports clearly demonstrate the presence of FABPs in the midgut epithelium of the investigated insect, hence suggesting a function in dietary lipid uptake (Table 2). FABPs have been identified in the midguts of the Lepidoptera *M. sexta* (MFB1, MFB2) (Smith et al. 1992), *B. mori* (*Bm*FATP) (Ohnishi et al. 2009), and *Spodoptera litura* (*Sl*fabp1) (Huang et al. 2012), as well as the Hymenoptera *Aphidius ervi* (*Ae*FABP) (Caccia et al. 2012). Nevertheless, further experimental investigation is needed to better understand their contributions to dietary lipids absorption. Once absorbed inside the midgut ECs, free fatty acids are further processed to either be oxidized in the mitochondria or used for the synthesis of TAGs, DAGs, and phospholipids. Lipids are mainly transported towards the peripheral storage tissues as DAGs bound to carrier proteins, called lipophorins, where they are subsequently stored as TAGs (Gondim et al. 2018). Fast handling of the absorbed dietary lipids helps to ensure low lipid concentrations inside the ECs, which creates a favorable concentration gradient promoting lipid diffusion across the membrane (Chapman 2013). Next to FABPs, other membrane-associated transporter proteins, namely the fatty acid transport proteins (FATPs) and the scavenger receptors class B type I belonging to the CD36 protein family, might be involved in the active transport of dietary fatty acids across the midgut membrane (Table 2). This expectation is mainly based on their observed fatty acid binding activities in peripheral tissues, for example in the silk glands (CD36) and the pheromone glands (FATP) of *B. mori* (Ohnishi et al. 2009; Sakudoh et al. 2010). However, no experimental data supporting their role in dietary lipid uptake across the midgut epithelium exist (Majerowicz and Gondim 2013; Miguel-Aliaga et al. 2018).

Since insects are unable to synthesize sterols de novo, their dietary absorption is of vital importance. Several mechanisms of sterol uptake have been proposed, involving both passive and active migration across the plasma membrane. Unfortunately, the molecular mechanisms behind dietary sterol absorption in insects are not yet fully elucidated. Based on vertebrate studies, two types of sterol-binding proteins have recently gained attention in insects: the Niemann-Pick C1 proteins and the sterol carrier proteins (SCPs) (Table 2). The Niemann-Pick C1 proteins are a well-defined family of transmembrane sterol-binding proteins in mammals, which are described to be critical for the cholesterol absorption in the intestine. Two members of the Niemann-Pick C1 family have been identified in the gastrointestinal tract of mammals, namely the Niemann-Pick C1 (NPC1), which is important for intracellular cholesterol transport, and the Niemann-Pick C1-like 1 (NPC1L1), which is important in general sterol absorption (Dixit et al. 2007). Two Niemann-Pick C1 genes were first identified in *D. melanogaster*. These closely related Niemann-Pick C1 homologs, called NPC1a and NPC1b, showed higher sequence similarity with NPC1 than with NPC1L1. NPC1b is only expressed in the midgut, while NPC1a has a wide, uniform tissue distribution. Functional characterization showed that NPC1b is responsible for the dietary cholesterol absorption inside the midgut epithelium, while NPC1a plays a role in intracellular sterol trafficking in the midgut and peripheral tissues (Voght et al. 2007; Zheng et al. 2018). More recently, the available genomes and transcriptomes of 39 insect species belonging to 10 different orders were analyzed for the presence of putative Niemann-Pick C1 genes. Similar to *D. melanogaster*, most studied insect genomes contained two *NPC1* genes, but a few had only one *NPC1* gene. Moreover, this research also indicated a gut-specific expression of *NPC1b* among the studied insects (Zheng et al. 2018). Another type of well-characterized sterol-binding proteins in vertebrates is the sterol carrier proteins (SCP2). The *SCP2* gene contains two distinct promoter sites, resulting in two different sterol carrier proteins: SCPx (sterol carrier protein-x) and SCP2 (sterol carrier protein-2) (Ohba et al. 1995). Some SCP2-encoding genes have been identified in insects, including *D. melanogaster* (Kitamura et al. 1996), *A. aegypti* (Krebs and Lan 2003), *S. littoralis* (Takeuchi et al. 2004), and *B. mori* (Gong et al. 2006). Tissue distributions of these identified insect SCPs suggest a mediating role in both the uptake and translocation of cholesterol. Moreover, RNAi mediated loss-of-function studies in *S. litura* have recently

demonstrated the role of SCP-x in the uptake of cholesterol across the midgut epithelium (Guo et al. 2009). Further functional characterizations of the above mentioned putative sterol-binding proteins are pivotal for understanding the precise mechanisms of dietary sterol uptake in insects.

Concluding remarks

Despite the intention of this review to provide a general overview of the most common strategies of insect macronutrient digestion and uptake, it should be clear that the way food is handled inside the gastrointestinal tract varies dramatically between different insect species. Most of this variation can be attributed to the diverse feeding strategies insects exhibit, ranging from herbivory to hematophagy. Furthermore, insects have also evolved numerous coping strategies to overcome different host defensive strategies, which are displayed by their overall large repertoire of digestive enzymes and defensive molecules. The ways in which insects internalize the degraded macronutrients from the intestinal lumen and translocate these throughout the body are far less documented; despite a consensus that the general mechanisms are analogous to those observed in vertebrates, there are as yet only a limited number of experimental studies supporting this theory. Consequently, more in-depth studies are essential to shed more light on the detailed mechanisms of nutrient uptake and allocation in different insects.

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Compliance with ethical standards

This article does not contain any studies with animals performed by any of the authors.

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