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Review paper

Genomics of adaptation to host-plants in herbivorous insects

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Abstract

Herbivorous insects represent the most species-rich lineages of metazoans. The high rate of diversification in herbivorous insects is thought to result from their specialization to distinct host-plants, which creates conditions favorable for the build-up of reproductive isolation and speciation. These conditions rely on constraints against the optimal use of a wide range of plant species, as each must constitute a viable food resource, oviposition site and mating site for an insect. Utilization of plants involves many essential traits of herbivorous insects, as they locate and select their hosts, overcome their defenses and acquire nutrients while avoiding intoxication. Although advances in understanding insect–plant molecular interactions have been limited by the complexity of insect traits involved in host use and the lack of genomic resources and functional tools, recent studies at the molecular level, combined with large-scale genomics studies at population and species levels, are revealing the genetic underpinning of plant specialization and adaptive divergence in non-model insect herbivores. Here, we review the recent advances in the genomics of plant adaptation in hemipterans and lepidopterans, two major insect orders, each of which includes a large number of crop pests. We focus on how genomics and post-genomics have improved our understanding of the mechanisms involved in insect–plant interactions by reviewing recent molecular discoveries in sensing, feeding, digesting and detoxifying strategies. We also present the outcomes of large-scale genomics approaches aimed at identifying loci potentially involved in plant adaptation in these insects.

Key words: plant–insect interactions; mechanisms; chemosensory genes; salivary genes; detoxification; plant cues; Hemiptera; Lepidoptera

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Introduction

Herbivorous insects are engaged in very intimate relationships with their host-plants, which constitute their food resource, mating site, oviposition site and habitat during all or part of their life cycles. This intimacy involves adaptations to cope with the plant phenology, specific nutrient composition and chemical/physical defenses. As a result, insect herbivores are often adapted to feeding on a restricted range of host-plants, which are related phylogenetically and/or by their biochemical composition [1–3]. In parallel, insects are known to adapt quickly to environmental changes, in particular exposure to pesticides, to which they frequently evolve resistance, and introductions of resistant cultivars and exotic plant species, which may eventually become part of their diet [4]. The constraints against the optimal use of a wide range of heterogeneous plant species, combined with the ability to adapt quickly to new hosts, probably explain why insect herbivores are the most species-rich lineages of eukaryotes. Indeed, nearly half of the approximately 1 million described insect species and a quarter of the known existing metazoan species comprise insect herbivores [5]. The hypothesis that herbivory increases speciation rates is supported by the higher species richness of specialist herbivores compared to generalist or non-herbivore-related taxa, and by the strong link between host shifts and speciation events in many herbivorous insect groups [3, 6, 7]. Occasional host-range expansions in specialists and the direct role played by plant adaptation on reproductive isolation between host-specialized populations are increasingly recognized as strong determinants of the diversification rates of herbivorous insects [3, 6, 8–10]. Therefore, unveiling the ecological, evolutionary and mechanistic bases of plant specialization in insect herbivores brings the promises of understanding the origin of a significant part of animal diversity and of developing more durable pest control strategies.

Although many important genes involved in plant–pathogen interactions have been identified through molecular and functional analyses [11], we are just starting to comprehend plant–insect interactions at the molecular level [12], in particular how insects overcome plant defenses and shift to new hosts [13, 14]. Here, we review the recent achievements made on the genomics of plant adaptation in two major insect orders, hemipterans (primarily aphids) and lepidopterans. Both groups include a large number of agricultural pest species for which genomic resources are now available [15–17]. This review focuses on how genomic and post-genomic studies have contributed to our understanding of the mechanisms involved in insect–plant interactions. First, we present recent discoveries in sensing, feeding, digesting and detoxification mechanisms of hemipterans and lepidopterans at the molecular level. Then, we consider 'without *a priori*' approaches that aim at identifying the genomic architecture of loci underlying adaptation of insects to their host-plants.

Molecular mechanisms of insect–plant interactions

Sensing of plant compounds

Chemosensation (olfaction and taste) plays a major role in insect–plant interactions [18], guiding insect preferences toward plants considered as good resources (for larval or adult stages) or as suitable oviposition sites (for mated females) [19, 20]. On one hand, plants emit volatile organic compounds (VOCs) and non-volatile molecules that insects use at long distance and at close range, respectively, to select an adequate host-plant. On the other hand, plants modify their emissions upon attack by insects. Some VOCs can repel herbivores, while others can attract their natural enemies.

The insect chemosensory organs mainly consist of antennae, proboscis and/or maxillary palps, depending on the insect order and developmental stage. The chemical recognition relies on the activation of a specific set of proteins, among which odorant-binding proteins (OBPs), olfactory receptors (ORs) and gustatory receptors (GRs) play a major role. The OBPs solubilize and transport chemicals, and contribute to the sensitivity of the chemosensory system [21, 22]. The specific activation of the chemosensory neurons on ligand binding, and thus the insect response, depends on the nature of the chemosensory receptor.

The loci controlling host recognition are far from being identified, but pioneering works on the evolutionary and functional mechanisms of chemosensation have been conducted in *Drosophila*, helped by the availability of complete sequenced genomes and the subsequent identification of large chemosensory gene families in this genus [23, 24]. These studies revealed that insect OBPs [25] and ORs/GRs [23] evolved rapidly via gene duplication/loss events, in parallel with adaptation to new ecological niches. Interestingly, the OBP repertoire has been shown to evolve more rapidly in specialized species (like *Drosophila sechellia* and *Drosophila erecta*) than in more generalist species [25]. Also, the sizes of OR/GR repertoires vary substantially between the 12 *Drosophila* sequenced genomes, and many of these gene families have undergone lineage-specific duplications [24]. One of the best examples supporting the importance of chemosensory genes in host specialization is *D. sechellia,* which has developed physiological and behavioral adaptations to its host-plant *Morinda citrifolia* [26]. The chemosensory system of *D. sechellia* has specialized in detecting key volatiles produced by *M. citrifolia* [27], and these insects have lost repellence in response to acids [28], possibly as a result of modifications of the expression level of some chemosensory genes, including OBPs [28, 29]. The *D. sechellia* lineage also shows accelerated gene loss and excess of coding changes at OR and GR genes [30]. However, whether these changes are adaptive to the narrow ecological niche of *D. sechellia* remains to be established by functional studies.

Rapid evolution of chemosensory genes has also been documented in herbivorous insect orders [31–34]. In lepidopterans, multiple OR/GR gene gains and a few gene losses have occurred during their evolution, and it has been hypothesized that gene family expansions of ORs and GRs have helped the adaptation of lepidopteran species to host-plants, following the angiosperm radiation [35]. For instance, in the specialist silkworm *Bombyx mori*, the GR gene family is characterized by a single large gene expansion of putative bitter receptors which are probably involved in the perception of a large variety of plant secondary chemicals that caterpillars and moths encounter [36]. In the passion vines butterfly *Heliconius melpomene*, another specialist, some GRs which have a female-biased expression in legs (which are equipped with gustatory sensilla used by females to select plant-specific oviposition site) show higher levels of gene duplication than the GRs expressed in both sexes, suggesting that female oviposition site preferences drive the evolution of GRs in lepidopteran genomes [37].

In the case of hemipterans, ORs and GRs have been annotated in the pea aphid genome, revealing that these groups of genes have undergone recent and rapid expansion through

aphid-specific duplications [38]. Although subject to several limitations and biases [39, 40], molecular tests of selection suggested that the most recently duplicated chemosensory genes in this species have evolved under positive selection, possibly in relation to the high level of host specialization observed in this aphid [38]. All these studies, however, only established correlations between genetic/transcriptomic differences and phenotypic differences related to host-plant specialization. Because the lineages or morphs used in the comparisons differ in other factors than just plant use, functional studies on candidate chemosensory genes, or host choice tests, are needed to test the effects of the observed genetic changes on host-plant adaptation.

Role of oral secretions in the response to plant defenses

Hemipterans are piercing-sucking insect pests feeding on plants by depleting phloem sap, but causing minimum mechanical damage on plants while feeding. As they require living cells to establish food ingestion, most of the hemipterans have a non-invasive penetration method using specialized mouthparts (stylets) involved in intercellular foraging [41]. This feeding behavior suggests that hemipterans have to suppress plant defense responses to facilitate phloem sap depletion. Aphids are the best studied hemipterans for their feeding strategies. When reaching sieve elements and ingesting phloem sap, aphids secrete two types of saliva: gelling saliva acts as a sheath protecting stylets from physical damage, and watery saliva (and particularly the proteins it contains) is injected into plant cells and may manipulate plant defense responses [42, 43]. Thus, aphid salivary proteins can be analogous to virulence factors of plant pathogens. Transcriptomic and proteomic analyses identified hundreds of proteins that can be secreted in aphid saliva [44–47]. Most of them have no predicted function and are specific to aphids.

In some aphid–plant interactions, aphid saliva or salivary proteins have been shown to suppress plant defense responses [47–50]. For instance, forisomes are calcium-dependent proteins involved in sieve element occlusion in response to aphid feeding. Aphid saliva contains calcium-binding proteins preventing forisome dispersion, allowing continuous flow of phloem sap [50]. In addition, it is hypothesized that plants produce toxic reactive oxygen species (ROS) in response to aphid feeding and that some aphid salivary proteins are involved in ROS detoxification [51]. Indeed, the Me23 salivary gene from the potato aphid *Macrosiphum euphorbiae* encodes a glutathione peroxidase and promotes aphid fecundity on *Nicotiana benthamiana* [52]. It has been proposed that glutathione peroxidase activity is involved in ROS detoxification, allowing the aphid to perform well on *N. benthamiana*. Another salivary protein, Mp55 from the green peach aphid *Myzus persicae* seems to suppress ROS production, as Mp55-transgenic *Arabidopsis thaliana* plants challenged with aphids induce less ROS production [49]. Moreover, Mp55 prevents callose deposition (involved in occlusion of sieve elements) and conversion of indol-3-yl-methylglucosinolate (I3M) to 4-methoxy-indol-3-yl-methylglucosinolate (4MI3M) [49], a deterrent glucosinolate for *M. persicae* [48]. In Mp55-transgenic *A. thaliana* plants, the level of I3M is higher than in control plants, confirming a lower level of I3M conversion to 4MI3M, which may promote aphid feeding [49]. *In planta* expressions of other aphid salivary genes (C002, PIntO1 and 2, Me10 and 23) increase aphid fecundity, but their respective roles in the suppression of plant defense responses against aphids are unclear [14, 52–54]. Interestingly, a recent work showed that *M. persicae* reproduction is increased when feeding on transgenic *A. thaliana* expressing *M. persicae*-specific salivary proteins (PIntO1 and 2) but not on plants producing the orthologs of these proteins from a legume specialist aphid (*Acyrthosiphon pisum*) [14]. The high rate of protein evolution of the genes encoding these proteins and signatures of positive selection on some codons of PIntO1 suggest a key role of these genes in host-plant adaptation and specialization [14]. The underlying molecular mechanisms of these salivary proteins remain to be identified.

Aphid saliva can also trigger plant defense responses [45, 46, 48, 55]. For example, a 3–10-kDa fraction of *M. persicae* saliva elicits defense responses against the aphid in *A. thaliana* [48]. Also, a salivary protein of *M. persicae*, named Mp10, shows a complex effect, as it both induces and suppresses plant defense responses. Indeed, Mp10 suppresses the oxidative burst and callose deposition triggered by the bacterial elicitor peptide flg22 while it triggers a chlorotic response in the plant and reduces aphid fecundity in *N. benthamiana* [47]. The Mp10 protein localizes in the host cytoplasm, suggesting that its plant targets localize in this compartment [56]. Another possible involvement of insect saliva in plant defense responses may be given by nucleotide binding site-leucine-rich repeats (NBS-LRR), a major class of plant resistance genes. A few NBS-LRR genes conferring resistance against hemipterans have been cloned: *Mi1-2* of tomato provides resistance to certain aphids, psyllids and whiteflies [57–59]. The *Vat* gene confers resistance to the aphid *Aphis gossypii* on melon [60, 61], while *Bph14* protects rice against the planthopper *Nilaparvata lugens* [62]. The incompatible interactions triggered by these resistance genes are often insect race- or biotype-specific. Although corresponding defense elicitors of the insects have not been identified yet, it is likely that the molecules secreted in insect saliva are recognized by the plants and cause incompatibility with the plants.

Unlike hemipterans, which are considered as 'stealthy' herbivores, lepidopteran larvae chew and ingest plant tissues and cause serious mechanical damage. Interestingly, plant responses to attacks by lepidopteran larvae present differences that may be accounted by oral secretions of the insects [63, 64]. Oral secretion of lepidopteran larvae is a mixture of saliva and regurgitant. So far, fatty acid-amino acid conjugates, glucose oxidase and inceptins, which are proteolytic products of plant chloroplastic ATP synthase γ-subunit, have been identified in lepidopteran oral secretions and shown to induce plant defense responses [65]. These elicitors are not general elicitors of plant responses to lepidopterans and specific interactions between the elicitors and plant species are suspected [65].

Lepidopteran oral secretion also contains proteins that suppress plant defense responses. The first reported oral effector was a glucose oxidase (GOX) identified in the saliva of *Helicoverpa zea*. This enzyme suppresses the production of nicotine in *Nicotiana tabacum*, which is triggered by wounding and caterpillar feeding, resulting in enhanced survival and weight gain of *H. zea* on tobacco [66]. A study of GOX activities in 23 families (85 species) of lepidopterans indicated that highly polyphagous species have higher levels of GOX activity relative to species with a narrower host range [67]. In the meantime, *H. zea* GOX triggers rapid defense responses in tomato, indicating that it acts as an effector or elicitor depending on the host-plant [68]. A suppressive activity of plant defense by lepidopteran oral secretion is also reported in the interactions between *A. thaliana* and two lepidopterans, *Pieris brassicae* and *Spodoptera*

littoralis [69]. In this study, the defense-suppressing molecule was not identified, but correlation between *S. littoralis* performance increase and the defense suppression by oral secretion was demonstrated. ATP hydrolyzing enzymes (apyrase, ATP synthase and ATPase 13A1) were also identified as saliva effectors of *H. zea* [70]. These enzymes suppress the induction of jasmonic acid and ethylene-regulated defense genes in tomato, but also inhibit the development of glandular trichomes, which are structures involved in herbivory defense. Whether these enzymes increase the performance of *H. zea* is not documented. Finally, an interesting study by [71] reported that the legume specialist caterpillar *Anticarsia gemmatalis* modifies the plant elicitor inceptin to produce an inactive and even antagonistic form, suggesting important key role of oral secretion in lepidopteran adaptation to host-plants.

Digestive system of insect herbivores in relation with plant use

As for feeding, the hemipterans and lepidopterans face contrasting digestion environments. At the larval stage, lepidopterans are able to ingest most parts of the host-plant (e.g. stem, leaf, flower and fruit) that contain proteins, lipids and major carbohydrates such as sucrose, starch, cellulose and hemicelluloses. By contrast, hemipterans feed on plant sap, which contains mainly carbohydrates and is low in proteins and amino acids [72]. Thus, digestion in hemipterans is believed to be mostly restricted to sugar dimer hydrolysis.

In lepidopterans, digestive enzymes have been identified from whole-genome analysis or from midgut proteomic or transcriptomic analysis [73, 74]. As expected from the high protein content of their diet, most digestive enzymes found in Lepidoptera are involved in proteolysis. Serine protease genes which are highly expressed in the midgut of *Plutella xylostella* [16] may circumvent the action of insecticidal plant protease inhibitors through differential expression in response to different plant hosts, as shown in *Helicoverpa armigera* [75]. Enzymes involved in sugar hydrolysis such as beta-fructo-furanosidases (also called sucrases or invertases) have been characterized in some lepidopteran species, e.g. *H. armigera* [73], *Manduca sexta* [76] and *B. mori* [77].

Because aphids feed on plant phloem sap poor in proteins, enzymes with proteolytic activity have received little attention until their identification in the midgut and in saliva [78, 79]. Furthermore, the analysis of the recently sequenced pea aphid genome revealed a very large expansion of genes encoding cathepsin B-like proteins, of the cysteine proteinase family, some copies of these genes evolving under positive selection and having a high expression level in the gut [80]. These genes may degrade the few ingested proteins to amino acids, as a supplementary source of nitrogen [79]. Alternatively, they may be involved in degradation of protease inhibitors produced by the host-plants [72]. Indeed, wheat proteinase inhibitors have been shown to have an anti-metabolic effect on the grain aphid (*Sitobion avenae*) midgut extract [81]. In a recent study, 5490 genes which are differentially expressed upon feeding on wheat have been identified in the grain aphid from a global transcriptome analysis of the insect alimentary canal, including genes involved in the production of precursor metabolites and energy, oxidation-reduction process and energy derivation by oxidation of organic compounds [82]. Comparative gut transcriptome analysis performed on two biotypes of the whitefly *Bemisia tabaci* species complex revealed a total of 5771 to 7000 midgut-specific genes, among which most had alpha-glucosidase, trehalose transporter and MFS transporter activities related to sucrose hydrolysis and nutrient absorption [83].

Although we now have a better view on the genes involved in digestion in hemipterans and lepidopterans, we acknowledge that we know very little on their actual roles in adaptation and specialization to the host-plant. However, we have examples beyond these two groups showing that few genetic changes in the digestive system may modify the ecological niche of a species, e.g. *Drosophila patchea* as obligate specialist on senita cactus [84].

Detoxifying mechanisms of plant secondary metabolites in herbivorous insects

In addition to the digestion of plant primary compounds, herbivores have developed enzymatic activities enabling them to metabolize plant secondary metabolites. Some species are also able to store and use plant toxic compounds for their own defense against predators and parasitoids [85, 86]. Genes involved in detoxification of plant secondary metabolites have been identified in various lepidopterans, allowing these species to occupy specialized ecological niches. For instance, the specialist butterfly *Heliconius sara* avoids the harmful effects of the cyanogenic leaves of *Passiflora auriculata* (passion vine) by converting cyanogenic glycosides to thiols and releasing nitrogen into the insect's primary metabolism [87]. On the same line, *P. xylostella* evolved genes involved in sulfate metabolism and encoding for glucosinolate sulfatase (GSS) and the associated sulfatase modifying factor 1 (SUMF1), enabling this moth to feed on various cruciferous plants (characterized by the glucosinolate-myrosinase defense system) by preventing the formation of toxic sulfates [16]. Instead of GSS, the Pieridae use the nitrile-specifier protein to detoxify glucosinolates in their host-plant. This molecular innovation seems to have evolved shortly (10 Myr) after the evolution of the host-plant group (Brassicales) [88], giving some evidence for co-evolution of insects and plants [3]. In the black swallowtail *Papilio polyxenes*, an inducible midgut cytochrome P450 with detoxifying activity allows larvae to feed on Rutaceae and Apiaceae, which both produce xanthotoxin, a highly toxic compound for generalist insect herbivores [89]. The effect of gossypol (a secondary metabolite found in the pigment gland of plants like cotton) on the generalist herbivore *H. armigera* was analyzed using transcriptomics [90]. When the insect was fed with the plant producing high gossypol concentrations, some genes (CYP6AE14 and CYP6AE11) encoding cytochrome P450 enzymes, as well as glucuronosyltransferases, carboxylesterases and a few glutathione S-transferases (GST), which are all enzymes involved in the degradation of xenobiotics, were predominantly over-expressed in the insect midgut. Silencing of CYP6AE14 confirmed the role of P450 enzymes in larval tolerance to gossypol [91]. Over-expression of specific P450 genes (CYP6AB9 and CYP9A17) was also observed in the gut of *H. armigera* depending on the plant structure eaten [92]. The tissue-specificity and level of expression of P450 genes of the fall armyworm, *Spodoptera frugiperda*, have been studied in response to various allelochemicals, allowing the identification of several P450s potentially involved in the plant adaptation of this polyphagous noctuid moth [93].

A global comparative analysis of the transcriptional response of the generalist *Heliothis virescens* and the specialist *M. sexta* to *Nicotiana attenuata* wild-type and defenseless transgenic plants (inactivated in nicotine production, TPI activity or jasmonic acid production) showed a greater number of genes involved in the regulation of plant secondary metabolism

(e.g. P450s, GSTs, carboxylesterases) when moth larvae were fed on defenseless plants [94]. *M. sexta* regulated specific genes according to the plant defense suppressed, while *H. virescens* regulated a large set of genes regardless of the defense suppressed. For example, larvae of the specialist *M. sexta* were more tolerant to nicotine than those of *H. virescens* (a smaller number of genes were regulated by nicotine in *M. sexta* than in *H. virescens*). Similar results were found when comparing polyphagous *H. armigera* and oligophagous *Helicoverpa assulta* gene expression profiles in relation to host-plant [95], supporting the hypothesis that herbivores can adapt to novel, toxic metabolites by becoming specialists [96].

For hemipterans, a key study compared three major types of enzyme involved in the detoxification process (P450 monooxygenases, GSTs and carboxyl/cholinesterases [CCEs]) between the legume specialist *A. pisum* and the generalist *M. persicae* [97]. This global comparison revealed an expansion of the P450 gene family in *M. persicae* with 115 genes identified (against 83 in *A. pisum*), which authors linked with the large host range of this aphid relative to *A. pisum*. No such expansion of the GSTs and CCEs was observed in either aphid species. However, as the complete genome of *M. persicae* is not available yet, the number of identified detoxification genes in this species is likely to be underestimated.

The cabbage aphid *Brevicoryne brassicae* and the turnip aphid *Lipaphis erysimi,* both specialized on brassicas, can sequester toxic glucosinolates from their host-plants into microbodies, yet avoid exposure to deterrent degradation products and gain protection to predation [98]. Also, GST enzymes in *M. persicae* have been shown to be involved in ingestion of isothiocyanates found in Brassicaceae, suggesting their role in allelochemical tolerance in this generalist aphid [99]. Furthermore, a work on the tobacco-adapted race (which is tolerant nicotine) of *M. persicae* showed an over-expression of a cytochrome P450 (CYP6CY3), which results from amplification of the gene and changes in its promoter [100, 101]. This suggests that changes in CYP6CY3 gene expression were responsible for a novel host-plant acquisition (here tobacco) in *M. persicae* and subsequent race formation.

Genomic studies of host adaptation without a priori

Comparative genomics of related lineages of herbivorous insects

De novo genome and transcriptome sequencing in lepidopterans and hemipterans opens new perspectives of research on the mechanisms underlying plant use and host specialization. The first analyses focused on global comparisons of genome structure among related species, relative to reference genomes (e.g. silkworm, pea aphid). These studies showed an overall gene synteny even between distantly related species, with the exception of local rearrangements [102–105]. While formal comparative genomics among plant-specialized species have not yet been performed in lepidopterans and hemipterans (as opposed to studies in *Drosophila* [106, 107]), patterns are emerging regarding gene composition and evolution, yielding new candidate genes or strengthening the role of candidates potentially involved in the evolution of insect host ranges. Sequencing the whole genome of *P. xylostella*, a species from an anciently diverged lepidopteran lineage, revealed an expansion of gene families associated with plant perception and detoxification of defense compounds [76]. Recent and multiple gene duplications in P450 genes were also observed in other lepidopterans (*S. frugiperda* and *H. armigera* [104]). At a more local genomic scale, a work by [108] reported a Z-autosome fusion that brought clusters of genes involved in detoxification of plant secondary metabolites in tortricid pest moths. This fusion may have significantly increased tortricid adaptive potential. In aphids, it has been proposed that the massive amplification of cysteine protease genes of the cathepsin B family described in *A. pisum* was responsible for the acquisition of novel alimentary modes through neo-functionalization or sub-functionalization of gene copies [109].

Qualitative comparative genomics also emerged from transcriptomic data, although the initial objective was examining gene expression level. For example, transcriptomic comparison of two moth species of the genus *Ostrinia* adapted to different host-plants identified, among the most divergent sequences, genes encoding for development, immunity and sensory functions [110]. Similarly, a large-scale transcriptomic analysis of the polyphagous *H. armigera* and the related oligophagous *H. assulta* highlighted differences in the number of genes regulating the chemosensory and detoxifying systems, which may account for their difference in host range [95].

While most of the examples in the previous sections reported genomic or expression patterns of genes previously known to be involved in sensing, feeding, digesting and detoxification, transcriptomics based on whole RNA sequencing may also reveal unsuspected functions. A recent work on *Polygonia c-album,* a polyphagous and widely distributed butterfly, showed large-scale transcriptional changes associated with the feeding plant (nettle or gooseberry), including variation of expression levels of serine-type endopeptidases, membrane-associated proteins, transporters, nucleic acid binding and, more surprisingly, of transcripts coding for structural constituents of the cuticle [111].

Comparative analyses between more or less distant host-affiliated lineages should be considered with some caution. First, they are not designed to test the actual role of host-plants in the genomic evolution of insects and thus may reveal genomic patterns that are unlinked to the host specialization process. Second, automated analysis of many genes may lead to erroneous interpretations due, for example, to errors during sequence alignment and annotation [39].

Genome scan of plant-adapted populations of herbivorous insects

Because many neurosensory, behavioral, morphological and physiological traits may contribute to plant use by insect herbivores, candidate gene approaches appear insufficient to decrypt the mechanisms underlying plant specialization. Moreover, insect species that have their genome annotated are distantly related, and thus differ by many more characteristics than their feeding strategies, making difficult to pinpoint the genomic loci controlling adaptation and specialization to distinct host ranges. Population genomic analysis of insect races or biotypes specialized on distinct sets of hosts can help to achieve that goal. Closely related lineages can be compared using genome scan methods, which are increasingly used to identify genomic regions potentially involved in adaptive divergence by screening genome-wide patterns of DNA polymorphism and differentiation to detect the locus-specific signature of positive directional selection [40].

This approach was applied to the lepidopteran *Ostrinia* species complex to detect genomic regions associated with plant

specialization and/or reproductive isolation. By comparing genetic differentiation at several hundred markers genotyped on populations from maize, hop or mugwort, a small percentage of the screened loci (so called 'outliers') displayed an elevated level of divergence among samples from different hosts, and were departing from neutral expectations [112, 113]. As no reference genome is available for *Ostrinia*, most of these outliers remain anonymous. By contrast, several studies that aimed at identifying the loci involved in plant adaptation in the pea aphid, which forms a complex of host-specialized biotypes, benefited from the genomic resources recently developed on this system, including a full annotated genome [17]. As previously mentioned, rapid expansion of GR and OR genes in the pea aphid lineage, along with recent evolution under positive selection for some of these genes, was evidenced for this aphid [38]. More recently, differences in copy number of certain genes of the GR and OR families were detected between pea aphid biotypes, outlining the potential importance of copy number variation in the adaptive process [114]. In addition, several GR and OR genes were shown to display higher differentiation in allele frequencies among biotypes than expected under the null hypothesis of neutral evolution [115], suggesting that different alleles at these genes are adaptive to distinct sets of plants. This result is consistent with patterns of differentiation at 390 microsatellite loci genotyped in the biotypes adapted either to alfalfa, red clover or pea. Among the 11 markers showing excessive levels of differentiation, two were located in the vicinity of OR genes and three others were near the genes coding for salivary proteins [116]. Four of these eleven outliers displayed high levels of differentiation among eight other biotypes, including the two markers located near OR genes [117]. However, one has to bear in mind that hundreds of genetic markers cover only a small percentage of the species' genome, and can reveal only a fraction of the loci that control host specialization. Genotyping-by-sequencing technologies allow much greater coverage and do not have such limitations. Still, an important issue of genome scans of wild populations pertains to the fact that these populations are in partial reproductive isolation and have different evolutionary histories. Hence, elevated genetic differentiation at certain loci can result from a range of extrinsic and/or intrinsic factors that may be functionally unrelated to host specialization, including genetic incompatibilities between populations, background selection and reduced recombination rates [118, 119]. In addition, genome scans tend to outline large genomic regions that often contain several hundreds of genes, only a few of which under putative host-induced selection. Reducing the number of candidates among these genes is difficult because a large fraction of aphid or lepidopteran genes are orphan and/or have unknown functions. This tends to bias investigations toward the few genes with known functions while neglecting other, potentially more interesting, candidates. Thus, genome scans of natural populations must be corroborated by independent lines of evidence, such as scans of laboratory populations under selection (e.g. in stick insects, [120]), QTL mapping of traits related to host use (see next section) and functional genomics.

Genetic architecture of insect traits involved in plant use and host specialization

Host-plant use by insect herbivores is a phenotypically complex trait that is likely to be polygenic, as it relies on a series of molecular mechanisms described in the first section. We know little about the genetic architecture of traits underlying plant use in insects, in terms of number of genes, chromosomal location and effects and interaction among quantitative loci [1, 121]. In the moth *Heliothis subflexa*, a specialist on *Physalis*, traits involved in plant use appear to be controlled by many loci of small effect scattered across different chromosomes [122]. In *P. xylostella*, adaptation to a novel host-plant (pea) was shown to have an oligogenic inheritance [123]. In aphids, QTL analyses of traits governing plant specialization have been performed on host races of *A. pisum*, adapted to either alfalfa or red clover. These studies revealed a complex genetic architecture of plant specialization. Notably, a positive genetic correlation between host acceptance and performance was found, likely to result from QTLs that either have pleiotropic effects on both traits or are closely linked on the chromosomes [121]. It also appears that the alleles adapting pea aphids to a given host-plant species are deleterious on other host-plants, a genetic antagonism that should prevent the evolution of a generalist genotype. More recently, a few QTLs controlling feeding behavior of specialized pea aphids on host and non-host-plants were identified, indicating an oligogenic basis of variation for this trait [124]. Interestingly, genomic regions showing elevated differentiation (relative to neutral expectations) between pea aphid host races tend to cluster around the QTLs for key traits involved in plant specialization (e.g. acceptance, performance) [125], suggesting that such regions create genomic islands from which further divergence may expand in later stages of speciation.

Conclusion

In recent years, candidate gene approaches, large-scale genome sequencing, transcriptomics, proteomics and genome-wide association studies have highlighted some mechanisms underlying plant adaptation in generalist and specialist herbivorous insects. Several key genes and their functions that are putatively involved in the various steps of plant use have been identified in lepidopterans and hemipterans, as summarized in Figure 1. Chemosensory genes are likely to be key components of host-plant recognition and choice by insects. An increasing number of insect proteins secreted into the plants are studied in respect to their role in suppressing or triggering plant defense reactions. Specific digestive and detoxifying enzymes may allow some herbivorous insects to adapt to new plant species by neutralizing harmful compounds. Several comparative genomic studies referenced here have outlined gene expansion as central in the evolution of insect host range. However, the phenotypic changes achieved by the evolution of gene copies or other genomic patterns deserve further investigations. Additional studies at macro-evolutionary scales are required to identify gene orthologies, convergence and parallel evolution, so as to link better patterns of genomic evolution to insect host ranges and feeding strategies. In parallel, experimental studies must be conducted in order to evaluate the adaptive nature of the traits controlled by the genes of interest. More importantly, functional analysis of these genes is needed as definitive proof of their involvement in plant selection and exploitation [126]. In particular, more functional validation of the signatures of positive selection detected through whole-genome survey should be provided. Indeed, tests for detecting selection and functional studies are too often decoupled. Functional analyses are conducted on a restricted set of pre-identified candidates mostly for feasibility reasons, while genome scans provide too many candidate genes for functional testing and present a high rate of false positives. Hopefully, new tools for functional validation are becoming accessible for non-model organisms and may bridge the gap between functional and genome scan studies. For instance, the genes of interest can be heterologously

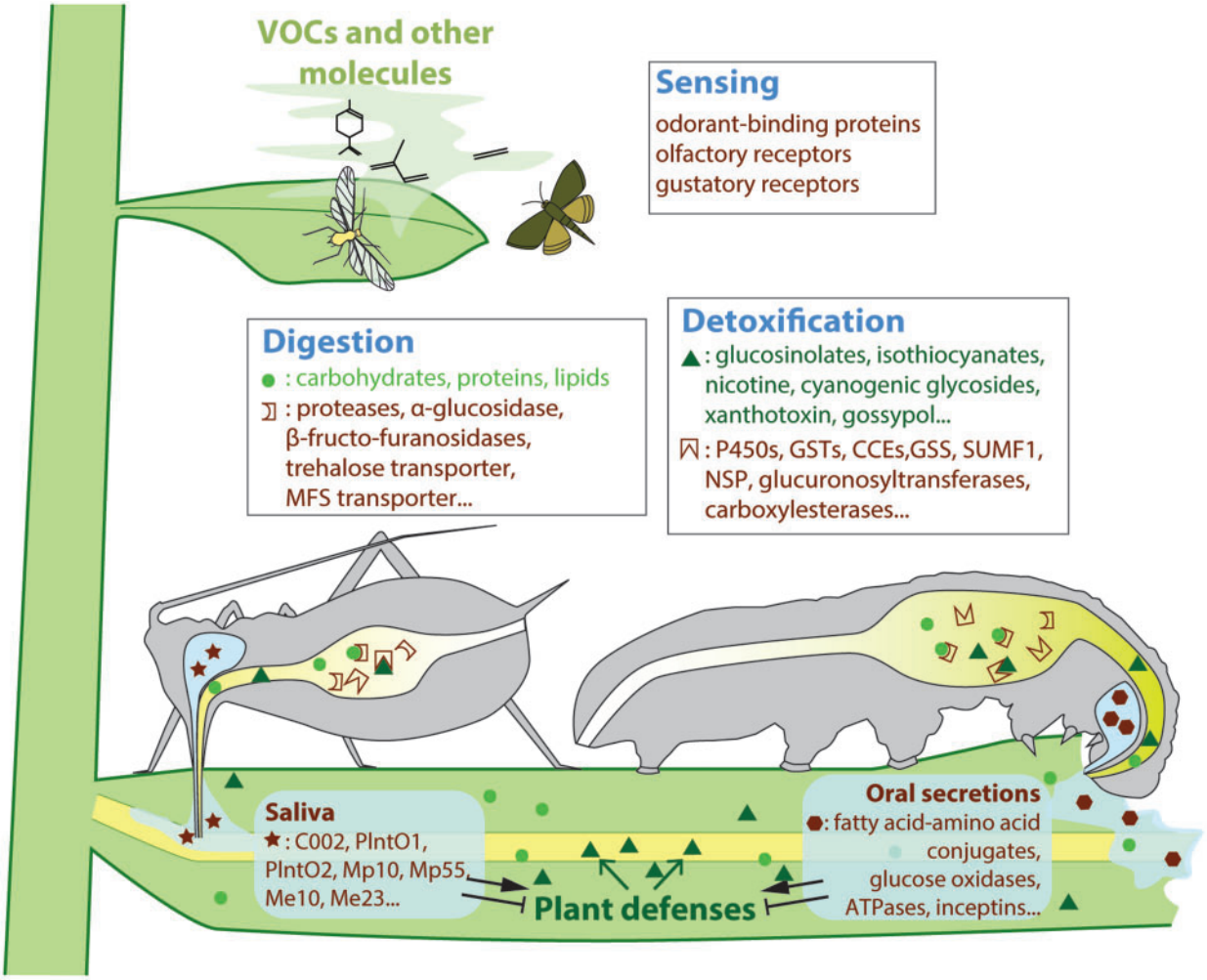

Figure 1. The major steps that are involved in the interactions between plants and herbivorous insects. Herbivorous insects sense plant VOCs and other molecules by odorant binding proteins, olfactory and gustatory receptors to select appropriate hosts. Hemipterans feed on plants using their stylets and cause minimum mechanical damage, while lepidopterans chew and ingest plant tissues and cause serious wounding. Both groups of insects secrete proteins and other molecules (brown stars or hexagons) into the plant and suppress or trigger plant defense responses which include production of toxic compounds (dark triangles). Several enzymes and transporters (square shapes with round indents) are reported to be involved in digestion and absorption of plant derived nutrients (light green circles). Plant derived toxic compounds can be degraded by insect enzymes (square shapes with triangle indents) or sequestered. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org.)

expressed and recombinant proteins produced for *in vitro* studies, such as binding studies for OBPs [127], ligand determination for chemosensory receptors [128], substrate identification and kinetic studies for enzymes. *In planta* expression of the proteins that may be secreted into plants allows characterization of their functions [47, 49, 52, 55, 56]. RNA interference appears feasible in aphids [129], while its efficiency in lepidopterans remains uncertain [130]. Although transgenesis is not yet established as a routine method in lepidopterans and hemipterans, promising genome editing methods based on new generations of nucleases coupled to DNA-recognition domains, such as zinc-finger nucleases, transcription activator-like effector nucleases or the clustered regularly interspaced short palindromic repeats (CrispR)/Cas9 endonuclease technology [131], will undoubtedly lead to efficient gene manipulation in these insect families.

In complement to their genome, most phytophagous insects host microbial communities that may expand their ability to exploit plants and modulate plant primary and secondary metabolisms and/or defenses against parasites [132–134]. Consequently, plant specialization of herbivorous insects may result from the outcomes of three-way interactions between plants, insects and their microbial associates. From an applied viewpoint, identifying the mechanisms underlying adaptation to host-plants in insects, including microbial partners as potential players, would offer new targets for the development of sustainable pest control strategies. Such an integrated vision will also help us understand how, and how fast, insects adapt to new host-plants and diversify, a process the details of which remain elusive more than 150 years after the publication of 'On the Origin of Species'.

Key points

- In recent years, candidate gene approaches, large-scale sequencing and genome-wide association studies highlighted some mechanisms underlying plant adaptation in non-model herbivorous insects.

- Several chemosensory genes, insect proteins secreted into the plant and digestive and detoxifying enzymes have been identified as involved in the various steps of plant use by herbivorous insects.
- Several comparative genomic studies also outlined gene expansion as central in the evolution of insect host range.
- Identifying the mechanisms underlying adaptation to host plant in insects would offer new targets for the development of sustainable pest control strategies and help us understand how and at what speed insects adapt onto new host plants and diversify

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