Sidestepping Darwin: horizontal gene transfer from plants to insects

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6   **Abstract**

7   Horizontal transfer of genetic material (HT) is the passage of DNA between organisms by means other
8   than reproduction. Increasing numbers of HT are reported in insects, with bacteria, fungi, plants and
9   insects acting as the main sources of these transfers. Here, we provide a detailed account of plant-to-
10   insect HT events. At least 14 insect species belonging to 6 orders are known to have received plant
11   genetic material through HT. One of them, the whitefly *Bemisia tabaci* (MEAM1), concentrates most
12   of these transfers, with no less than 28 HT events yielding 55 plant-derived genes in this species.
13   Several plant-to-insect HT events reported so far involve gene families known to play a role in plant-
14   parasite interactions. We highlight methodological approaches that may further help characterize
15   these transfers. We argue that plant-to-insect HT is likely more frequent than currently appreciated
16   and that in-depth studies of these transfers will shed new light on plant-insect interactions.

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18   **Highlights**

19   • Recent HGT detection workflows enable large-scale studies of HGT among eukaryotes
20   • At least 14 insect species belonging to 6 orders contain plant-derived genes
21   • Many plant-derived genes have putative functions involved in plant-parasite interactions
22   • The impact of plant-to-insect HGT on insect evolution remains to be characterized

23

Introduction

Darwin proposed that evolution occurs through natural selection of intraspecific variations transmitted across generations [1]. But he was unable to provide a satisfactory explanation for the origin of these variations and the mechanism underlying their transmission. Darwin and Mendel were contemporaries, but they never met and Mendel's work was ignored by Darwin [2]. About 50 years after Darwin and Mendel died, Darwin's natural selection and Mendelian inheritance, which posits that individuals inherit a combination of alleles from their parents, became two pillars of the modern synthesis. Remarkably, observations made in bacteria as early as 1928 – *i.e.*, before the modern synthesis was initiated – suggested that genetic information could also be transmitted between individuals through horizontal transfer (HT), *i.e.*, through means other than vertical, parent-to-offspring, inheritance [3]. In the second half of the 20$^{\text{ieth}}$ century it became apparent that HT of genes was rampant in prokaryotes and that it was a major source of bacterial innovation [4]. The first hint that HT of genetic material was not restricted to prokaryotes but could also occur in eukaryotes may correspond to the discovery of endogenous retroviruses in vertebrate genomes during the late 1960s and early 1970s [5]. It was then suggested that the endosymbiotic origin of eukaryotes was accompanied by the relocation (through HT) of many organellar genes to the nuclear genome [6], and that transposable elements (TEs) [7,8], as well as non-organellar genes [9,10] could be acquired horizontally in these taxa. These pioneering works were followed by the observation that the genome of phagotrophic single-cell eukaryotes contained many genes captured from their prokaryote and microbial eukaryote preys [11–13]. Today, the importance of HT of genes in recent eukaryote evolution remains a matter of debate [14–18]. But as a matter of fact, we are witnessing an increasing number of HT reports in both uni- and multi-cellular eukaryotes, largely fueled by the many high-quality new whole genome sequences that are deposited on a daily basis in public databases [19–24]. Importantly, many of these transfers likely facilitated the adaptation of the receiving species to a new ecological niche [25–27]. In multicellular eukaryotes, most HT of genes reported so far involve genes of bacterial, fungi, or viral origin [23,25,28–31]. Aside from HT of TEs which are not rare [32], relatively few HT of genes between multicellular eukaryotes have so far been uncovered. This trend is seemingly changing in plants, in which numerous recent cases of plant-to-plant HT have been characterized [33–36]. In vertebrates, a single such event is known so far, which involves transfer of a gene coding an antifreeze protein between the Atlantic herring and the rainbow smelt [37]. Here, we provide a comprehensive account of another type of eukaryote-to-eukaryote HT, namely plant-to-insect HT, highlight methodological challenges inherent to the detection of such transfers, and discuss the possible mechanisms underlying these HT as well as their impact on insect genome evolution and plant insect interactions.

How many genes of plant origin in insect genomes?

The first claim of plant-to-insect HT was published in a 2015 article in which hundreds of transcripts from the mosquito *Anopheles culicifacies* were reported to encode proteins 100% identical to proteins found in plants [38]. However, using plant proteins listed in supplementary table 1 of Sharma et al., (2015) [38], we were unable to recover plant-derived genes in the genome of *A. culicifacies*, which is now available in Genbank (accession number: AXCM00000000.1). Two cases of TE transfer between plants and arthropods have been described but in both studies, HT was deemed more likely to have occurred from arthropod to plants rather than in the opposite direction [39,40]. The first robust case of plant-to-insect HT is thus the one reported in Lapadula et al., (2020) [41], in which a gene of plant origin encoding a ribosome inactivating protein (RIP) has been acquired at least 80 million years ago by an ancestor of two whitefly species (*Bemisia tabaci* and *Trialeurodes vaporariorum*). Whiteflies (family Aleyrodidae; Hemiptera) are important agricultural pests as they are able to feed upon a large spectrum of host plants and they can transmit many viruses to plants [42]. After transfer from an unknown plant donor, the RIP gene was duplicated once and twice in the lineage leading to *B. tabaci* and *T. vaporariorum*, respectively. Quickly following this report, two other plant-derived genes encoding BAHD acyltransferases were characterized, again in the whitefly *B. tabaci* [43].

These studies prompted us to perform a systematic search for plant-to-*B. tabaci* HT, which yielded no less than 49 plant-derived genes in the genome of the Middle East Asia Minor 1 (MEAM1) whitefly [44]. We found that these genes could be clustered in 24 orthogroups, 20 of which have members in another whitefly cryptic species (Sub-Saharan Africa - East and Central Africa [SSA-ECA]), which diverged at least five MYA from the MEAM1 whitefly [45]. We uncovered another two plant-derived genes in *T. vaporariorum*, which diverged several dozens of MYA from *B. tabaci* [46], but we were unable to firmly conclude on whether these genes were acquired from plants independently in this species or whether they were transferred in an ancestor of *B. tabaci* and *T. vaporariorum*.

In parallel to our study, a large-scale, systematic survey of HT performed by Li et al. (2022) [31] in 218 insect species uncovered no less than 1410 foreign genes. While the vast majority of these genes have a bacterial source, many were transferred from fungi and viruses and a total of 43 were assigned a plant origin. Just by itself, *B. tabaci* accounts for 24 of these 43 genes, most of them being also reported in our study (see next section). The reason why *B. tabaci* has a much higher number of plant-derived genes than other insects is not immediately evident but we note that this species also has a higher number of genes transferred from bacteria and the higher overall number of horizontally acquired genes (170 versus 68 in *Contarinia nasturtii*, the species with the second higher number of foreign genes). Whether horizontal gene flux is higher in *B. tabaci* or whether it is similar to other

91   insects but foreign genes have a higher chance to be retained in *B. tabaci* remains to be assessed.

92   Interestingly, Li et al. (2022) [31] also uncovered plant-derived genes in three other species of

93   Hemiptera, one Coleoptera, three Diptera, one Hymenoptera, one Orthoptera and two Thysanoptera.

94   In total, and accounting for the redundancy between our results [44] and those of Li et al. (2022) [31]

95   for *B. tabaci*, a total of 156 genes of plant origin have been so far uncovered in the genome of 14 insect

96   species belonging to six orders (Figure 1). It is noteworthy that nine plant-derived genes are annotated

97   as plant transposons in *Aedes* mosquitoes [31], begging the question of whether these genes

98   correspond to recently acquired TEs that may still be able to transpose or whether they arise from

99   molecular domestication of ancient plant TEs horizontally acquired by an ancestor of *Aedes*

100   mosquitoes.

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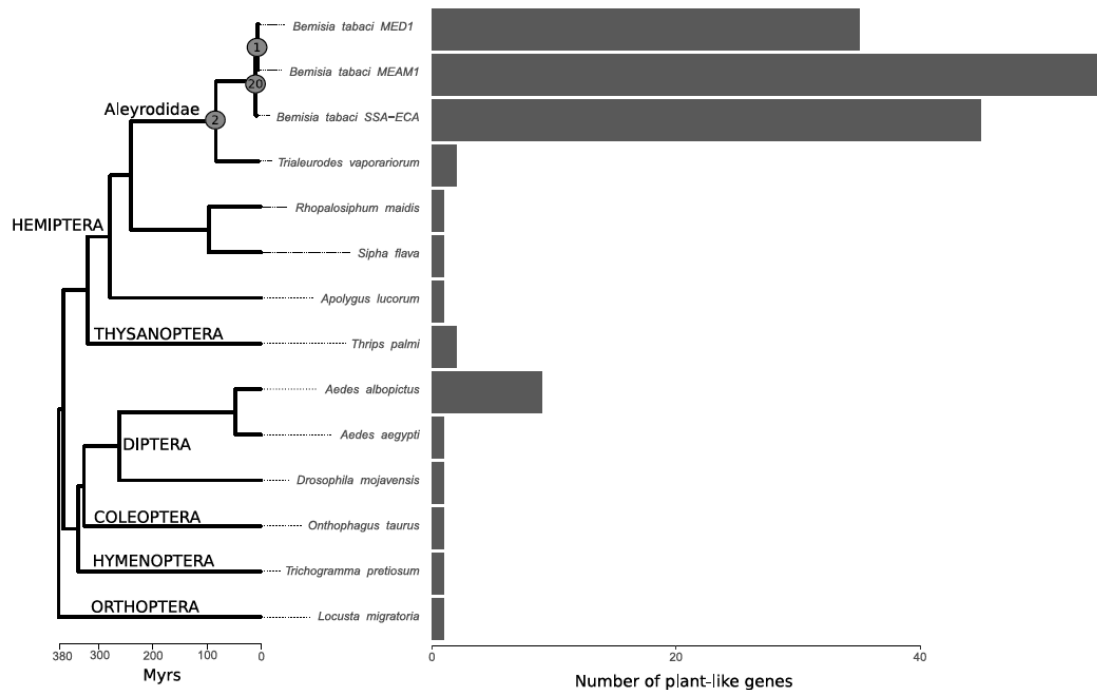
Figure 1. Number of plant-derived genes identified in insect genomes. The number of plant-derived genes in *Bemisia tabaci* MEAM1 results from our merging of results from [31] and [44]. Details regarding which genes were found in which study and on the predicted function of these genes are given in Supplementary Table 1. The tree and divergence times were taken from REF. 46 and 79. Numbers at nodes in Aleyrodidae correspond to putative numbers of horizontal transfer events that took place in the ancestors of Aleyrodidae, as inferred in [44] based on the delineation of orthogroups. Nine of the plant-derived genes from *Aedes* mosquitoes are annotated as encoding plant transposon proteins in supplementary table 2 of [31].

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Methodological challenges to detect horizontal gene transfer

Inferences of HGT are plagued by methodological issues and several hypotheses alternative to HGT must be considered before HGT is favored [44,47,48]. It is for example essential to check that what looks like a horizontally acquired gene is not instead a contaminant. This may be done using fluorescent *in situ* hybridization, as in Dunning Hotopp et al. (2007) [49] or PCR as in Li et al. (2022) [31]. Another way to discard the possibility of contamination is to find orthologs of horizontally acquired genes in a group of species, the genome assembly of which were produced independently in different labs. Other recommendations on HGT validation have been discussed in previous papers [44,47,48,50]. Yet, even when greatest care is taken, large-scale studies of HGT may yield different results due to differences in the bioinformatics approach being implemented. This is particularly well-illustrated by the two recent reports of HGT from plants in *B. tabaci* (MEAM1) [31,44]. Both studies used overall similar workflows, being based on sequence similarity search between *B. tabaci* query proteins and a target database followed by the analysis of the taxonomic distribution of hits to identify candidate HT proteins. Homologs of each candidate were then retrieved and used to build phylogenetic trees that were manually examined for HGT validation. However, the two workflows present key differences (Figure 2). First, they rely on different target databases. Li et al. (2022) [31] used Refseq proteins from NCBI (approx. 240 million proteins as of September 2022, https://www.ncbi.nlm.nih.gov/refseq/statistics/) combined with the proteomes from the 218 insects they studied. Instead, Gilbert & Maumus (2022) [44] used UniRef90 from Uniprot (https://www.ebi.ac.uk/uniprot/TrEMBLstats), which is composed of representative proteins from UniRef100 clustered at 90% (152 million proteins as of November 2022 being representative of 318 millions).

Furthermore, the two studies differ in how they call HGT candidates from the result of similarity searches. In Li et al. (2022) [31], an « alien index » (AI) is calculated, which was first introduced in Koutsovoulos et al. (2022) [51], and broadly used to select HGT candidates [e.g. 50]. The AI, computed for each HGT candidate, measures the extent to which the best hit against targets from the query taxonomic group (ingroups) differs from that obtained against targets from other groups (outgroups). To limit the number of false positives, Li et al. (2022) [31] also calculated the percentage of outgroup species among the top hits. This percentage will be low if the homolog is not conserved in outgroups or if the gene family is comparably distant to homologs across several taxa. By contrast, Gilbert & Maumus (2022) [44] have used an original approach in which the UniRef90 target database is depleted of ingroup proteins (here Aleyrodidae) to infer the last common ancestor (LCA) of each query protein. Using this taxonomic exclusion, the placement of a protein LCA in Viridiplantae (plants) occurs when a significant proportion of its best homologs are plant proteins, suggesting a plant origin. The study

applied three different modes of LCA inference implemented in the taxonomy module of the MMseqs2 program [52], in which the taxonomical assignment of the LCA can be inferred using two methods derived from the 2bLCA protocol [53] or using lowest common ancestor of equal-scoring top hits (this last mode being theoretically similar to AI-based measures). To focus on most robust HGT candidates, only the proteins with LCA in Viridiplantae below the genus level were considered as a filter attesting for their conservation in plants.
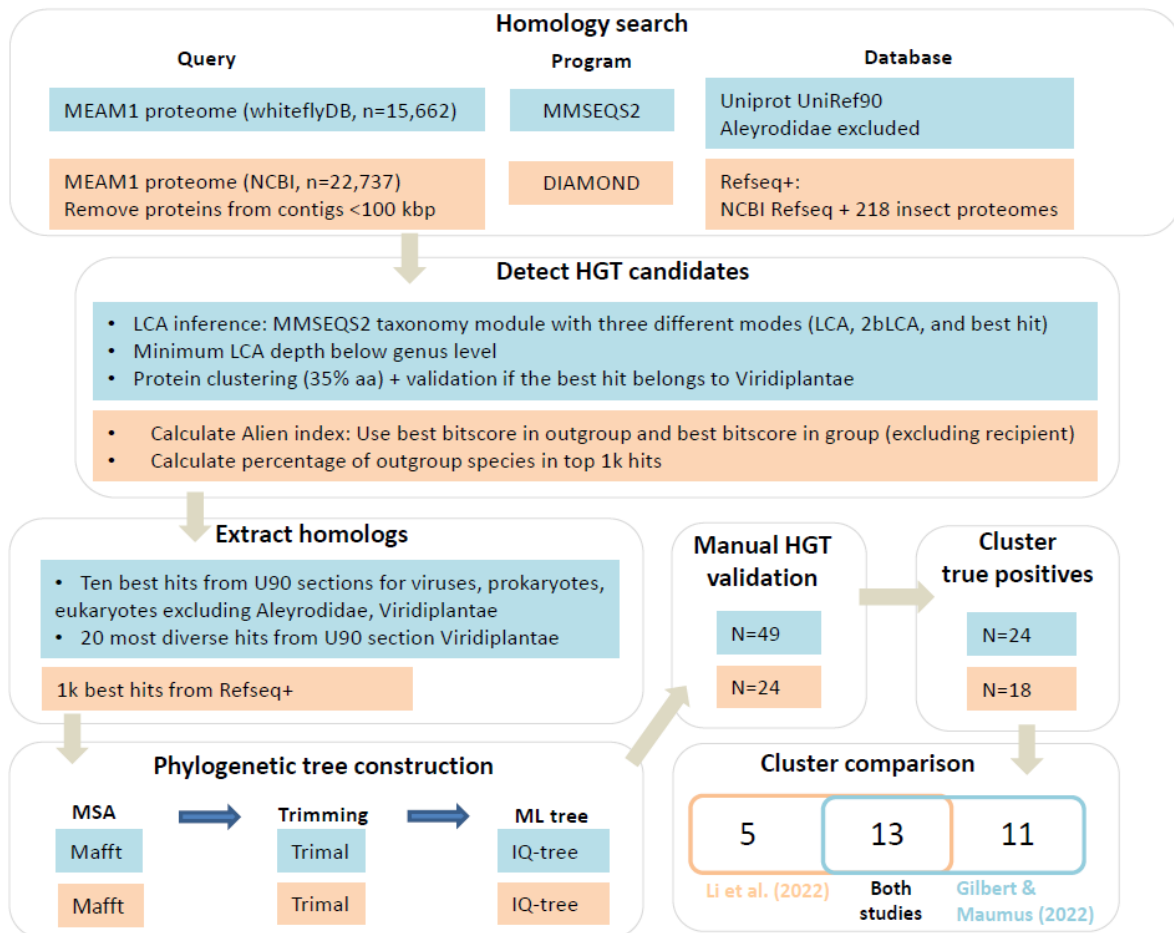
Figure 2. Graphical comparison of the HT detection workflows used in Li et al. (2022) [31] and Gilbert & Maumus (2022) [44]. The main steps of the workflows are separated in grey boxes and the methods used at each step by REF. 31 and REF. 44 are indicated in salmon and blue rectangles, respectively.

Using deep protein clustering (35% identity), Gilbert & Maumus (2022) [44] found that the 49 plant-derived proteins from *B. tabaci* MEAM1 correspond to 24 clusters. Here, we applied the same clustering to the 24 plant-derived proteins from Li et al. (2022) [31], which resulted in 18 clusters. We found that only 13 clusters comprise proteins from the two studies while 11 and 5 are unique to REF. 44 or REF. 31, respectively (Figure 2 and Supplementary Table 1). One cluster unique to REF. 31

comprises two genes (XP_018917389.1 and XP_018909952.1) for which we could not confirm plant origin. Two other clusters unique to REF. 31 have no homolog in the MEAM1 proteome used in REF. 44. This comparison shows that the HT detection workflow we used in REF. 44 is more sensitive, but it is not entirely comprehensive. Understanding the causes of reciprocal false negatives will help improving the sensitivity of HT detection. This comparison also shows that the number of insect HGT reported in REF. 31 may be largely underestimated and that HGT, including from plants, may have had an even stronger impact on insects than what is apparent from this study.

Functional impact of plant genes in insects

Only one experimental study characterized the function of a plant-derived gene in insect [43]. Together with the predicted function of all plant-derived genes in *B. tabaci*, it suggests that much like bacteria-to-insect HGT [54], plant-to-insect HGT was a source of important new functions to insects, which may have facilitated adaptation to their environment. REF. 43 showed that the protein encoded by the *BtPMaT1* plant-derived gene in *B. tabaci* has malonyl-transferase activity, that is, it catalyzes the transfer of a malonyl group onto several phenolic glucosides, which are secondary compounds produced by many plants to defend themselves against herbivorous insects. Malonylation detoxifies phenolic glucosides and renders them harmless to whiteflies. In agreement with this, whiteflies feeding on transgenic tomato plants that express small interfering RNAs targeting *BtPMaT1* are unable to survive [43]. Thus, whiteflies harnessed a plant gene which allows them to thwart plant defenses and was perhaps essential in the evolution of these hemipterans towards becoming generalist herbivores [43,55].

In addition to this functional study, indirect evidence including evolution under purifying selection and expression suggests that most if not all other plant-derived genes in *B. tabaci* encode important functions [41,44]. To help explore these putative functions, we provide a table compiling predicted functions of all *B. tabaci* plant-derived proteins reported in Li et al. (2022) [31] and Gilbert & Maumus [44] (Supplementary Table 1). Several of these proteins have predicted functions previously shown to play a role in plant-parasite interactions and response to pathogens, not necessarily insects but rather fungi and oomycetes. It remains unclear however if and how these genes enabled whiteflies to adapt to different host plants. The protein functions inferred are based on the annotation of homologs and only speculative. Only three plant-derived gene clusters have a potentially evident role in helping the insect bypassing host defenses. These include *BtPMat1* [43] as well as a predicted plant cell wall degrading enzyme (PCWDE) and a predicted alpha-(1,4)-fucosyltransferase which could be involved in cell wall patterning [56]. Perhaps more surprisingly, many plant-derived genes have putative functions linked to the production and activation of plant defenses. For instance, plant-derived genes annotated

as BAHD acyltransferase and phenylalanine ammonialyase have putative functions in the production of phenolic secondary metabolites [57], many being defense compounds. That said, the *B. tabaci* plant-derived BAHD acyltransferase has best BLAST hits against Dcr-like (Defective in Cuticular Ridges) homologs. Dcr is not involved in production of phenolic compounds but is instead required for the assembly of cutin polymers, which are the major components of the outermost layer of plant defense, the cuticle [58,59]. The whitefly plant-derived genes also comprise homologs of pathogen-related proteins (which are produced by plants upon biotic attack) and proteins that have known roles in plants in pathogen recognition and/or defense signaling pathways including Thaumatin [60], subtilisin-like protease [61] or glucan endo-1,3-beta-glucosidase [62]. An advantage to herbivory could generally make sense if the encoded proteins are inactive and simply tether plant enzymes or occupy receptors to limit the defense response. It is also possible that some plant-derived genes may be recycled by insects to protect themselves against pathogens, parasites or predators. In agreement with this scenario, the RIP genes reported in REF. 41 are toxic N-glycosidases playing a role in plant defense against pathogens and insect predators [63]. As pointed out by Lapadula et al., the whitefly RIPs might play a role similar to those produced by *Spiroplasma* endosymbionts in *Drosophila*, known to protect their host against nematodes [64] and parasitic wasps [65].

Other plant-derived genes in *B. tabaci* may have predicted functions in nutrient and micronutrient assimilation, glycoprotein secondary modifications, and fatty-acid desaturation including a large family of delta(12) fatty acid desaturases (Supplementary Table 1). It will be interesting to develop experimental approaches to characterize these genes and how they may have been beneficial to whiteflies compared to genes acquired from bacteria [54]. In this context, testing the function of the *B. tabaci* plant-derived pectinesterase should be relatively straightforward as it has a clear predictable function in degrading plant cell walls. It would also be worth addressing whether the catalytic sites in predicted plant-derived enzymes are conserved to assess their catalytic potential and which plant-derived genes are specifically expressed in the insect saliva or gut to suggest a direct link with herbivory.

How can plant DNA be transferred to insects?

The mechanisms underlying HT in eukaryotes remain poorly understood. That most foreign genes so far reported in eukaryotes are from prokaryotes (this is especially true for insects [31,66]) may in part be due to the capacity of several bacteria to transfer their DNA to eukaryotic cells through conjugation [67]. Furthermore, many arthropod and other metazoan species harbor obligate or facultative intracellular symbionts [68], some of which are in close contact with the host germline genome, facilitating HT [30]. The higher proportion of foreign prokaryotic versus eukaryotic genes so far

227 uncovered in eukaryote genomes may also have methodological underpinnings. Transfers are easier
228 to infer between distantly related species (*e.g.* between prokaryotes and eukaryotes) than between
229 more closely related ones (*e.g.* between two eukaryotes). Systematic studies focusing on detecting
230 eukaryote-to-eukaryote HT remain scarce. In any case, this later type of transfer is more difficult to
231 explain than prokaryote-to-eukaryote transfers because no mechanism dedicated to HT is known in
232 eukaryotes (but see [69]). Plant-to-plant HTs have been proposed to occur accidentally through whole
233 genome transfer in heterospecific grafts [70], illegitimate pollination, haustoria in parasitic plants [71],
234 or root-to-rhizome contact [33]. Regarding plant-to-insect transfers, one might argue that the close
235 ecological interactions existing between plants and most insects facilitate HT. Indeed, all insect species
236 in which plant-derived genes have been found so far (Figure 1) are herbivorous or nectarivores.
237 Whether DNA can be transferred from plants to insects through feeding, as proposed in the case of
238 bacteria-to-single cell eukaryotes HT [11] remains to be tested. In this context, it is noteworthy that
239 insects can internalize dsRNA produced by plants through feeding [43,72], so one could speculate that
240 DNA from their food may also occasionally penetrate into insect cells. However, the « you are what
241 you eat » hypothesis may not be fully sufficient to explain plant-to-insect HT as the majority of
242 herbivorous insects included in REF. 31 are devoid of plant-derived genes. Another, non-mutually
243 exclusive hypothesis is that vectors such as exosomes or viruses are involved in plant-to-insect HT
244 [73,74]. If true, the known exposure of *B. tabaci* to a very large diversity of plant viruses, several of
245 which are known to replicate in whitefly cells [75], may in part explain the higher number of foreign
246 genes found in this, compared to other insect species [31,44]. In this context, it is noteworthy that
247 whiteflies are the main insect vectors of dozens of Begomovirus species, which are highly successful
248 plant viruses. Some of these circular single-stranded DNA viruses are able to replicate in insect cells,
249 persist during the entire lifetime in the insect and can undergo transovarial transmission [76].
250 Acquisition of foreign genes by *B. tabaci* under the form of DNA, rather than RNA, through the
251 intermediate of DNA viruses is consistent with the fact that most plant-like genes uncovered in
252 whiteflies contain at least one intron [44]. However, it is also possible that these genes were
253 transferred through an RNA-mediated mechanism and later acquired introns once in the *B. tabaci*
254 genome.

Conclusions and outstanding questions

256 The first plant-to-insect HGT was reported recently [41] and since then 156 plant-derived genes have
257 been found in 14 insect species from six orders. The reasons why the whitefly *B. tabaci* concentrates
258 most of these genes, as well as most non-plant foreign genes among insects, are unknown. Based on
259 a comparison between two recent studies [31,44], we contend that the number of horizontally
260 acquired genes in insects, including those acquired from plants, is likely strongly underestimated. In

261   addition to improve current HGT pipelines that rely on the use of annotated protein-coding sequences,

262   it would be interesting to perform more comprehensive HGT searches using whole genomes. This

263   would allow assessing whether the currently observed higher number of horizontally acquired plant

264   genes versus plant TEs in insect genomes has biological underpinnings or is merely due to

265   methodological limitations. Should the current trend hold, it would be in striking contrast with the

266   trend observed for insect-to-insect HTs, as most if not all currently known such events involve TEs and

267   not genes [77]. Future large-scale examination of plant-to-insect HGT may also shed light on the factors

268   possibly influencing such transfers and on whether some plant and/or insect life history traits correlate

269   with HT numbers. Of particular relevance will be to identify recent plant-to-insect HGT events, as this

270   may help identifying donor plants species and the mechanisms underlying transfer. A deeper

271   understanding of the impact of plant-to-insect HGT will only be reachable through multiple functional

272   studies of insect plant-derived genes, such as the one performed in REF. 43. In addition to help

273   decipher the intricacy of plant-insect interactions, such studies have the potential to unveil new targets

274   for controlling insect pests, which may in some contexts be less problematic than pesticide-based

275   strategies [78].

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277

278 **Legends of additional elements**

279 **Supplementary Table 1. Annotation of MEAM1 plant-derived proteins found in REF. 31 and REF. 44.**

280 The alternating shades of grey distinguish the clusters established at 35% amino-acid sequence identity

281 using MMseqs2. The false positive cluster from Li et al. (2022) is not indicated. The Genbank protein

282 identifiers begin with "XP" while the WhiteflyDB identifiers begin with "Bta". The annotations

283 correspond to the transfer of annotations of the best hits obtained by BLAST against Genbank. The

284 functional category and putative function are only speculative.

285

286 **Acknowledgements**

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*Together with ref 43, this study provides a proof of principle that horizontally acquired genes may be good targets to develop new strategies to control insect pests. The impact of such strategies on the environment however remains to be assessed compared to pesticide-based approaches.

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