

## **How has HGT shaped the evolution of insect genomes?**

Chao Liu<sup>1,2</sup>, Yang Li<sup>1</sup>, Yun Chen<sup>1</sup>, Xue-xin Chen<sup>1</sup>, Jianhua Huang<sup>1</sup>, Antonis Rokas<sup>3</sup>,  
and Xing-Xing Shen<sup>1,2\*</sup>

<sup>1</sup>College of Agriculture and Biotechnology and Centre for Evolutionary & Organismal Biology,  
Zhejiang University, Hangzhou 310058, China

<sup>2</sup>Zhejiang Lab, Hangzhou 311121, China

<sup>3</sup>Department of Biological Sciences and Evolutionary Studies Initiative, Vanderbilt University,  
Nashville, TN 37235, USA

\*Correspondence: xingxingshen@zju.edu.cn (X.X.S.)

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## Summary

As the most diverse group of animals on Earth, insects are key organisms in ecosystems. Horizontal gene transfer (HGT) refers to the transfer of genetic material between species by non-reproductive means. HGT is a major evolutionary force in prokaryotic genome evolution, but its importance in different eukaryotic groups, such as insects, has only recently begun to be understood. Genomic data from hundreds of insect species have enabled the detection of large numbers of HGT events and the elucidation of the functions of some of these foreign genes. Although quantification of the extent of HGT in insects broadens our understanding of its role in insect evolution, the scope of its influence and underlying mechanism(s) of its occurrence remain open questions for the field.

Insects are a highly diverse lineage comprising >50% of all described animals, with about 30 orders (Novotny et al., 2002; Chapman et al., 2009; Forister et al., 2019). Insects are found in most terrestrial and aquatic environments (Gullan and Cranston, 2014; Scudder, 2017) and are key for ecosystems' health in a variety of ways, such as by acting as decomposers, prey, predators, and pollinators (Gurr et al., 2003; Majeed et al., 2022). In addition, their relatively small sizes and their high reproductive rates allow them to occupy diverse ecological niches that larger creatures cannot (Berger et al., 2008; Gullan and Cranston, 2014).

Insects also interact extensively with microorganisms, such as bacteria and fungi, enhancing insects' abilities to adapt to different environments. Microorganisms are found in the exoskeleton, gut, and blood of insects, as well as inside insect cells. The insect gut microbiota contributes to the host's digestion and provides essential amino acids and vitamins. Some microbes can also synthesize specific toxins that boost insect immunity and protect the insects against pathogens (Engel and Moran, 2013; Douglas, 2015). In addition, several chemical signal molecules produced by microbes can even regulate insect social behavior such as courtship and kinship recognition (Engl and Kaltenpoth, 2018).

Horizontal gene transfer (HGT) refers to the transmission of genetic material between organisms by means other than sexual reproduction (Ochman et al., 2000; Keeling and Palmer, 2008; Dunning Hotopp, 2011). HGT overcomes the mating barriers between organisms and can be an important source of genetic innovation (Keeling and Palmer, 2008; Fitzpatrick, 2012; Burmeister, 2015; Wisecaver and Rokas, 2015; Husnik and McCutcheon, 2018). HGT is very common in bacteria but occurs more rarely in eukaryotes. It is estimated that 1.6 - 32.6% of genes in a bacterial genome has been acquired by HGT (Koonin et al., 2001). However, the small number of multicellular eukaryotic genomes makes it difficult to estimate how many genes have been acquired

by HGT in eukaryotes. With the advances of sequencing technologies, genomics and bioinformatics have substantially changed our understanding of HGT in eukaryotes. In the past two decades, large numbers of HGT events have been reported (Panoff and Chuiton, 2004; Wickell and Li, 2020). Now it appears that HGT in eukaryotes occurs more often than what we expected and has contributed to adaptation of a wide variety of organisms.

The mechanisms by which HGT occurs are well known in bacteria but are less well understood in eukaryotes. In bacteria, the mechanisms of HGT include transformation, transduction, and conjugation (Arnold et al., 2022). Once exogenous DNA has entered a cell, it can be incorporated into chromosomes by homologous recombination (Li and Heyer, 2008). In eukaryotes, HGT can occur through a variety of vectors, such as symbiotic microorganisms, viruses or transposons (Catoni et al., 2018; McDonald et al., 2019; Perreau and Moran, 2022). Symbiotic microorganisms are currently thought to be the main vectors of foreign DNA in eukaryotes (Li et al., 2022; Perreau and Moran, 2022). The degree of openness of the symbiotic relationship between host and symbiont largely determines the ease and frequency of HGT occurrence (Perreau and Moran, 2022). Host genetic population structure, which can be classified as open, closed, or mixed, can influence foreign gene transmission and retention. Host-symbiont genetic recombination activity is common in open and mixed symbiotic relationships.

HGT-acquired genes across different insect orders exhibit diverse functions (Figure 1). For example, in Diptera, a large amount of genetic material from *Wolbachia* bacterial endosymbionts has been integrated into the genome of *Drosophila ananassae*. This foreign DNA was involved in the evolution of metabolic pathways in *Drosophila* (Dunning Hotopp et al., 2007). In Lepidoptera, most butterflies and moths have acquired exogenous genes from bacteria; one of these transferred genes was shown to

enhance the courtship of male moths (Li et al., 2022). Horizontally acquired genes could also enhance the recipient insect's defenses. A parasitoid killing factor gene acquired from a virus to lepidopterans contributes to defense against natural enemies (Gasmi et al., 2021). In Coleoptera, herbivorous beetles can use plant cell wall degrading enzymes (PCWDEs), which were horizontally obtained from bacteria and fungi, to convert complex fibers of plant cell walls into simple oligosaccharides and monosaccharides (McKenna et al., 2019; Kirsch et al., 2022). In Hemiptera, whiteflies have acquired the plant-derived phenolic glucoside malonyltransferase gene *BtPMT1*. This foreign gene enables whiteflies to neutralize toxins in plants. This suggests that herbivores use genetic material from their host plants to evolve resistance to plant defenses (Xia et al., 2021). Finally, carotenoid biosynthesis genes transferred from fungi to pea aphids contribute to a red-green color polymorphism, which influences their susceptibility to natural enemies (Moran and Jarvik, 2010).

A recent study systematically examined HGT across 218 insect genomes (Li et al., 2022), finding that HGT is widespread in insects and that the donors of the transferred genetic material are diverse. 80% of exogenous genes originated from bacteria, with smaller proportions derived from complex eukaryotes, including fungi (11%) and plants (4%) (Li et al., 2022). Did these HGT events occur via direct eukaryotic-eukaryotic contact or is there a prokaryote that acted as an intermediate vector to assist their transfers? We do not know, but no matter which hypothesis turns out to be correct, the prerequisite of DNA material transferred into eukaryotic organisms from other prokaryote or eukaryote donors is that its transmission should occur in eukaryotic germ cells. Otherwise, these HGT-acquired genes will not be passed on to offspring in insects. In addition, Li et al., found that the number of HGT events in insects varied widely in different orders (Li et al., 2022). For example, the numbers of HGT-acquired genes in insects of the orders Hymenoptera and Diptera are relatively small, whereas the number of HGT-acquired genes is much higher in the order Lepidoptera. What factors lead to disproportional distribution of HGT across major insect orders? Although the answer

to this question remains a mystery, consideration of the ecologies of insects in different orders might be helpful to answer it. Lastly, it is unclear whether microorganisms, such as symbionts, acquired foreign genes from insects via HGT.

In summary, many previous studies have found the occurrence of HGT in insects, but there are some burning questions that still are unknown:

- i.** How did HGT occur between insects and their donors?
- ii.** Why are microorganisms the major donors?
- iii.** Why are lepidopterans more prone to HGT?
- iv.** Does HGT ever occur in the opposite direction, i.e., from microorganisms to insects?

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## **Declaration of Interests**

The authors declare no competing interests.

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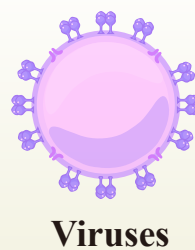
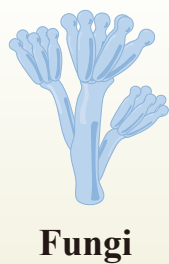
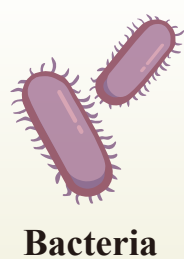
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## Figure legend

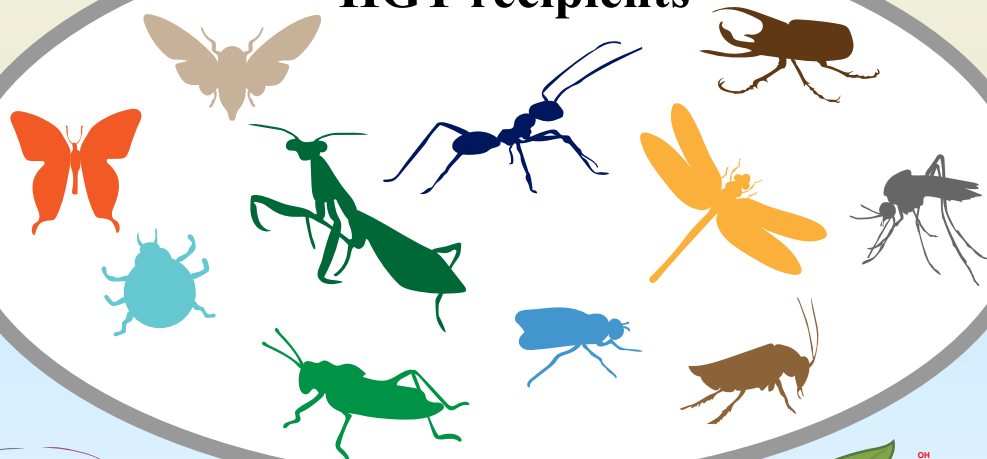
**Fig 1. Insect genes horizontally acquired from bacteria, fungi, viruses, and plants are responsible for diverse insect adaptations.** Some well-known examples of HGTs are shown as follows: carotenoid biosynthesis genes transferred from fungi to pea aphids contribute to a red-green color polymorphism (Moran and Jarvik, 2010); genes that neutralize phenolic glucosides acquired from plants contribute to the detoxification

capabilities of whiteflies (Xia et al., 2021); a parasitoid killing factor gene transferred from a virus to lepidopterans contributes to lepidopteran defense (Gasmi et al., 2021); plant cell wall degrading enzymes (PCWDEs) obtained from bacteria and fungi contribute to efficient plant tissue digestion (McKenna et al., 2019; Kirsch et al., 2022); and an HGT-acquired gene from bacteria enhances male courtship behavior in lepidopterans (Li et al., 2022).

## HGT donors



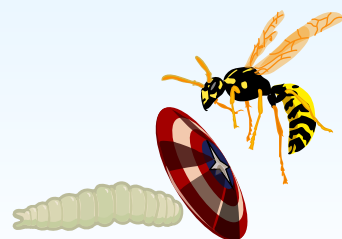
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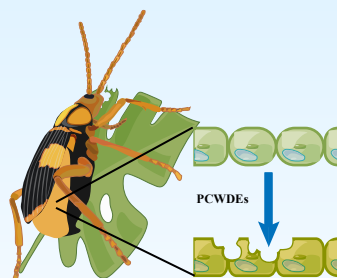
## Diverse adaptations



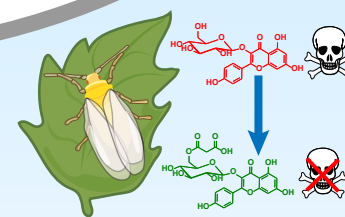
Changing body colour



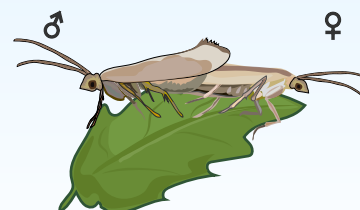
Enhancing defence



Degrading plant cell wall



Detoxifying compounds



Boosting male courtship