



Fig. 1 Example of wild flower margin in one of our site. Photo: Vincent Doublet.

rect ‘contact networks’ among pollinators (via the use of flower species). Particularly, we found flower diversity to be positively correlated with the reduction of niche overlap between insect species. This response of insect pollinators suggest that wild flower margins with high plant species richness may reduce insect competition for resources, and potentially reduce the risk of inter-specific disease transmission by supporting diverse diet for insects exploiting different flowers.

To test the effect of plant diversity on pathogen dynamics in bees, we sampled pollinators on these farms and characterized their virome by deep transcriptome sequencing. We are now combining these environmental data to virus discovery in order to reveal the impact of the agri-environmental scheme on viral dynamics. Ultimately, we aim to identify environmental (flower density, agricultural practices) and ecological factors (plant taxa, insect community assemblage) that significantly enhance the transmission of plant and pollinator viral diseases within our model to eventually improve agricultural practices and wildlife management.

Disclosure of interest The authors declare that they have no competing interest.

References

- [1] M.E. Craft, Infectious disease transmission and contact networks in wildlife and livestock, *Phil. Trans. R. Soc. B.* 370 (2015) [20140107].
- [2] M.A. Fürst, D.P. McMahon, J.L. Osborne, R.J. Paxton, M.J.F. Brown, Disease associations between honeybees and bumblebees as a threat to wild pollinators, *Nature* 506 (2014) 364–366.
- [3] R. Manley, M. Boots, L. Wilfert, Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors, *J. Appl. Ecol.* 52 (2015) 331–340.

<https://doi.org/10.1016/j.crv.2019.09.013>

13

Microbial nutrient factories in insects on extreme diets

Angela E. Douglas

Department of Entomology, Cornell University, Ithaca, NY, USA

E-mail address: aes326@cornell.edu

Insects are renowned for their capacity to specialize on a wide diversity of diets, many of which are nutrient-poor or nutritionally unbalanced. For example, various insects feed through the life cycle on wood, vertebrate blood, plant sap and other extreme diets that are variously deficient in vitamins, sterols and essential amino acids. These insects circumvent the fundamental “rules” of animal nutrition because they

possess symbiotic microorganisms that overproduce the limiting dietary nutrients. Many associations between insects and microorganisms are evolutionarily ancient and involve the exquisite coevolution of metabolic function in the insect and microbial partners, including the restructuring of microbial metabolism as nutrient factories for the host (Fig. 1). These insects include major pests and vectors of animal, human and crop disease agents. Their dependence on specific microorganisms offers novel routes for the control of these globally important insect pests.

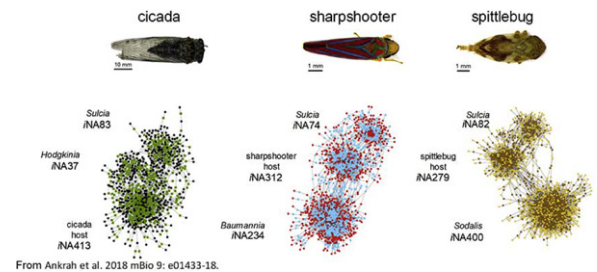


Fig. 1 Metabolic networks in xylem feeding insects.

Disclosure of interest The author declares that she has no competing interest.

Further reading

N.Y.D. Ankras, B. Chouaia, A.E. Douglas, The cost of metabolic interactions in symbioses between insects and bacteria with reduced genomes. *mBio* 9 (2018) e01433-18.

N.Y.D. Ankras, A.E. Douglas, Nutrient factories: metabolic function of beneficial microorganisms associated with insects. *Environ. Microbiol.* 20 (2018) 2002–2011.

A.E. Douglas, The multi-organismal insect: diversity and function of resident microorganisms, *Annu. Rev. Entomol.* 60 (2015) 17–34.

<https://doi.org/10.1016/j.crv.2019.09.014>

Session IV. Interaction with other organisms

14

The insect reservoir of diversity for viruses and antiviral mechanisms

Jean-Luc Imler

Unité “Modèles insectes d’immunité innée” (M3i), Institut de biologie moléculaire et cellulaire du CNRS, Université de Strasbourg, France

E-mail address: jl.imler@ibmc-cnrs.unistra.fr

Insects originated more than 400 million years ago and have undergone since then an extraordinary diversification, associated with many spectacular innovations, such as flying or establishment of social societies. They have colonized all terrestrial ecosystems, and are exposed to a broad range of pathogens, including viruses, bacteria, fungi, and parasites. Like all animals, insects rely on innate immunity to control infections. Innate immunity is the first layer in host-defense in animals. It involves receptors sensing the presence of infectious microorganisms and triggering signaling that leads to the expression of genes coding effector molecules, which concur to counter the infection. In vertebrates, a subset of genes induced encode cytokines and coreceptors that activate a second layer



of host defense known as adaptive immunity. The study of the innate immunity in insects has led to (i) the discovery of antimicrobial peptides, which target bacteria and fungi and are now known to be present in all animals and in plants, (ii) the identification of evolutionarily conserved important genes activating innate immunity, e.g., the Toll-like receptors, and (iii) a better understanding of viral and parasitic diseases transmitted by hematophagous vector insects.

Among infectious microbes, viruses represent a particular threat because they offer few intrinsic targets for inhibition by antiviral molecules. This is because they consist in their simplest form in a nucleic acid encapsulated in a protein shell, and hijack molecular machineries from host cells to complete their replication cycle. Of note, recent advances in high-throughput sequencing (HTS) technologies have opened the way to the characterization of the virome (i.e. the genetic diversity of viruses in a biological sample) in insects. Interestingly, these studies revealed that (i) infection by one or more viruses is common in arthropods, (ii) the genetic diversity of arthropod viruses surpasses that described previously, and (iii) the genetic diversity of viruses found in plants and vertebrate animals fall within the genetic diversity of viruses associated with arthropods [1].

This suggests that arthropods may have participated in the evolution of viruses causing human disease and points to the relevance of characterizing antiviral mechanisms in insects.

One reason to investigate insect–virus interactions is that hematophagous insects, for example *Aedes* mosquitoes, are vectors of important viral diseases such as Zika, dengue, yellow fever, and chikungunya. RNA interference is an RNA-based mechanism that offers broad protection against viruses in insects. This elegant mechanism relies on the recognition of double-stranded (ds) viral RNAs by the RNase III enzyme Dicer-2, which processes them into 21 nucleotide long small interfering (si) RNA duplexes. One strand of the siRNA is then loaded onto the RNase H-like enzyme Argonaute 2, where it serves as a guide to specifically target viral RNAs. Of note, virus-derived siRNAs, which provide a footprint of the action of the insect immune system, can be characterized by HTS. In collaboration with the group of Prof. Joao Marques (UFMG, Belo Horizonte, Brazil), we have shown that small RNA sequencing allows assembling longer contigs of viral RNAs, generating a better coverage of viral genomes, than traditional long RNA sequencing. In addition, the profile of the small RNAs is characteristic of the virus from which they derive (e.g., number of reads, size distribution of the small RNAs). For example, some viruses infect the ovaries and generate a different type of small RNAs, known as Piwi-interacting RNAs, or piRNAs, which are longer (24–28nt) than siRNAs (Fig. 1). As a result, it is possible to assign a viral origin to sequences even if they do not exhibit any homology to sequences present in the databases. This represents a significant improvement in the characterization of the insect virome [2]. We have applied this strategy to wild *Aedes* mosquitoes collected in a dengue endemic region in Brazil, and have identified three viruses. Two of them, the Bunyavirus Phasi Charoen-like virus (PCLV) and the unclassified virus Humaita-Tubianga virus (HTV), have a high prevalence in *Aedes* mosquitoes collected in different regions of Brazil. These poorly characterized viruses may affect the dynamics of transmission of known viral pathogens such as dengue, Zika,

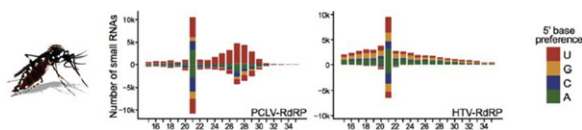


Fig. 1 Profiles of small RNAs generated from two viruses identified in wild *Aedes aegypti* mosquitoes in Brazil. PCLV, which can be found in ovaries from female mosquitoes, generates both siRNAs and piRNAs, whereas HTV only generates siRNAs. The sequences analyzed map to the viral RNA-dependent RNA polymerase (RdRP). Data are from ref. [2].

or chikungunya viruses, a hypothesis that is currently being tested in the laboratory.

The discovery of the important role played by Toll-like receptors in innate immunity revealed that important gene regulatory networks have been conserved during evolution and illustrated how studies in insects can lead to important findings for the biomedical field. This has provided strong incentives to identify and characterize other evolutionarily conserved host-defense mechanisms. As a consequence, the contribution of non-conserved genes to insect immunity has received less attention. Yet, these genes may be just as important as the conserved genes. Indeed, insects evolved independently of mammals for several hundreds of thousands of years, which provided multiple opportunities to develop original strategies of defense against infections. Hence, the characterization of insect-specific antiviral factors may inspire new strategies to counter infections. For example, we recently characterized the gene *diedel*, which is strongly induced following viral infection in *Drosophila*, and has been hijacked at least three times by insect DNA viruses. We showed that *diedel* encodes a circulating protein that suppresses the activity of the immune deficiency (IMD) pathway of host-defense. The IMD pathway is one of the two major innate immunity pathways regulating transcription factors of the NF- κ B family in flies. The discovery that several insect viruses have hijacked a cellular gene suppressing the IMD pathway prompted us to investigate its contribution to the control of viral infections. Interestingly, we discovered that two components of the pathway, the kinase IKK β and the NF- κ B transcription factor Relish, are required to restrict infection by two picorna-like viruses,

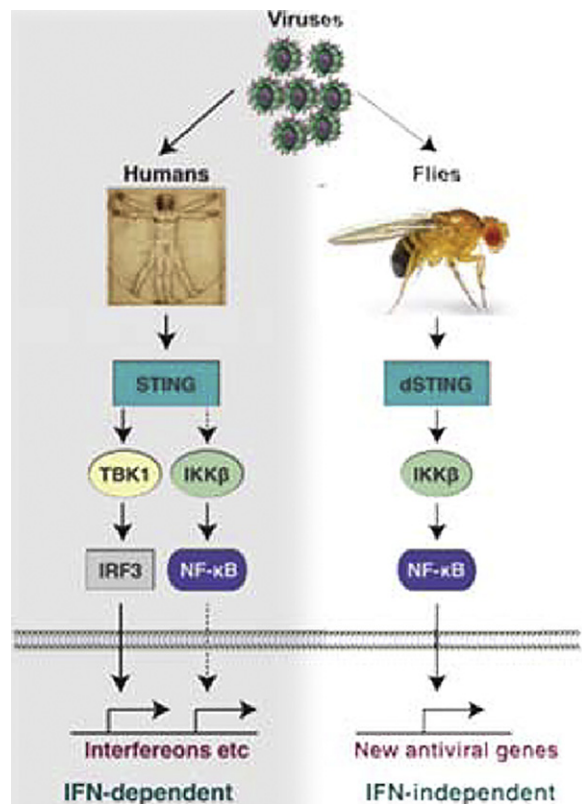


Fig. 2 A STING-IKK β -NF- κ B pathway controls an inducible antiviral response in *Drosophila*. STING is an evolutionarily conserved gene regulating antiviral innate immunity. In vertebrates, including humans, it triggers the production of interferons, which regulate the expression of factors restricting viral infection. In flies, STING regulates expression of a set of genes, including the antiviral factor Nazo.

Drosophila C Virus (DCV) and Cricket Paralysis Virus (CrPV) in *Drosophila*. By contrast, the other components of the pathway, including the regulatory subunit of the IKK β kinase, NEMO, do not appear to play a role in the resistance to infection by these viruses. Among the genes regulated by IKK β in virus-infected flies, we identified two genes involved in the resistance to viral infection. The first one is the homologue of the mammalian factor STING (Stimulator of Interferon Genes), and we could show that it acts upstream of IKK β and Relish in a new signaling pathway (Fig. 2). The second one encodes a new antiviral factor, that we called Nazo (meaning “enigma” in Japanese) [3]. The STING-IKK β -Relish signaling cassette controls inducible expression of Nazo in response to viral infection. Nazo results from a duplication of the gene CG3740 in *Drosophila* species from the *Sophophora* subgenus. Of note, CG3740 is not upregulated by viral infection, and ectopic expression of the gene has no effect on replication of DCV or CrPV, unlike expression of Nazo, which results in strong suppression of viral replication. The discovery of Nazo provides an excellent opportunity to decipher the genetics by which a cellular gene acquires a new function in antiviral immunity. Furthermore, the characterization of its mode of action against picorna-like viruses may reveal novel angles of attack against a family of viruses that include many serious human pathogens (e.g., poliovirus). In summary, the fantastic diversity of insects extends to the viruses they carry, and to the genetic mechanisms they evolved to control these viruses. This biodiversity provides a unique opportunity to extend the repertoire of known antiviral mechanisms and to identify weak spots in the replication cycles of viruses.

Disclosure of interest The author declares that he has no competing interest.

References

- [1] M. Shi, et al., Redefining the invertebrate RNA virosphere, *Nature* 540 (2016) 539–543.
- [2] E.R. Aguiar, et al., Sequence-independent characterization of viruses based on the pattern of viral small RNAs produced by the host, *Nucl. Acids Res.* 43 (2015) 6191–6206.
- [3] A. Goto, et al., The kinase IKK β regulates a STING- and NF- κ B- dependent antiviral response pathway in *Drosophila*, *Immunity* 49 (2018) 225–234.

<https://doi.org/10.1016/j.crv.2019.09.015>

15

Cassava mealybug biological control delivers multi-faceted societal benefits

Kris Wyckhuys

China Academy of Agricultural Sciences, China

E-mail address: kagwyckhuys@gmail.com

As core component of sustainable intensification, biological control constitutes a tailor-made solution for the long-term management of both endemic and invasive pests. Here, drawing upon the example of the cassava mealybug (*Phenacoccus manihoti*; Hemiptera), we illuminate the myriad benefits of insect biological control.

In 2008, the above mealybug invaded Asia, where it inflicted a 27% drop in aggregate cassava production and triggered a 260% surge in starch prices. Mealybug-induced shocks in Thailand's cassava output were offset by a 300,000-ha expansion of the agricultural frontier—accompanied with major loss of intact tropical forest. Mealybug outbreaks were permanently resolved through the release of the neotropical wasp *Anagyrus lopezi* (Hymenoptera). This minute parasitoid effectively suppressed *P. manihoti* at a continent-wide scale, restored food security, and delivered pest control services worth US\$ 200–700/ha. This same parasitic wasp had previously averted famine for ~ 20 million people in Africa. Our work emphasizes how beneficial (pest-controlling) insects

help meet food production needs while benefiting farmers' pockets, global commodity trade, and the environment.

Disclosure of interest The author declares that he has no competing interest.

Further reading

K.A. Wyckhuys, W. Zhang, S.D. Prager, D.B. Kramer, E. Delaquis, C.E. Gonzalez, W. Van der Werf, Biological control of an invasive pest eases pressures on global commodity markets, *Environ. Res. Lett.* 13(9) (2018) 094005.

K.A.G. Wyckhuys, A.C. Hughes, C. Buamas, A.C. Johnson, L. Vasseur, L. Reymondin, J.-P. Deguine, D. Sheil, Biological control of an agricultural pest protects tropical forests, *Commun. Biol.* 2 (2018); <https://doi.org/10.1038/s42003-018-0257-6>.

K.A. Wyckhuys, P. Wongtiem, A. Rauf, A. Thancharoen, G.E. Heimpel, N.T. Le, M.Z. Fanani, G.M. Gurr, J.G. Lundgren, D.D. Burra, L.K. Palao, Continental-scale suppression of an invasive pest by a host-specific parasitoid underlines both environmental and economic benefits of arthropod biological control, *PeerJ.*, 6 (2018) e5796.

<https://doi.org/10.1016/j.crv.2019.09.016>

16

Insects and their microbial partners: The *Drosophila* case study

François Leulier

Institute de génomique fonctionnelle de Lyon, École normale supérieure de Lyon, University of Lyon, France

E-mail address: francois.leulier@ens-lyon.fr

Metazoans establish reciprocal interactions with their commensal bacterial communities. Despite recent progress, a clear view of the physiological benefits associated with host/microbiota relationship remains elusive. Hence the molecular mechanisms through which the microbiota exerts its beneficial influences are still largely undefined. In this line, we aim at deciphering the molecular dialogue governing the mutualistic interaction between intestinal bacteria and their host. To



Fig. 1 *Lactobacillus plantarum* (associated with blue food and feces) colonizes the host intestine (in both adults and larvae) and is transmitted vertically to progenies (note the contamination of an egg being laid) and promotes larval growth (note the size difference of *L. plantarum* associated individuals (larvae with blue intestine) vs. non colonized larvae (no blue in the intestine) when grown for six days on a low nutrient diet). © François Leulier.