Evolutionary Immunology to Explore Original Antiviral Strategies

Jean-Luc Imler [1,2], Hua Cai [2], Carine Meignin[1], Nelson Martins[1,*]

ORCID information : JLI (0000-0003-0740-8319) ; HC (0000-0002-6697-652X) ; CM (0000-0002-6588-9045) ; NM (0000-0002-3923-2998)

[1]Université de Strasbourg, CNRS UPR9022, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

[2]Sino-French Hoffmann Institute, School of Basic Medical Science, Guangzhou Medical University, Guangzhou, China

*Present address: Instituto Gulbenkian de Ciência, Oeiras, Portugal; Católica Biomedical Research Centre (CBR), Católica Medical School, Universidade Católica Portuguesa, Oeiras, Portugal

Correspondance: jl.imler@ibmc-cnrs.unistra.fr

Keywords : innate immunity ; STING ; cyclic dinucleotide ; cGAS ; CBASS

ABSTRACT

Over the past 25 years, the field of evolutionary developmental biology (evo-devo) has used genomics and genetics to gain insight on the developmental mechanisms underlying the evolution of morphological diversity of animals. Evo-devo exploits the key insight that conserved toolkits of development (e.g., *Hox* genes) are used in animals to produce genetic novelties that provide adaptation to a new environment. Like development, immunity is forged by interactions with the environment, namely the microbial world. Yet, when it comes to the study of immune defence mechanisms in invertebrates, interest primarily focuses on evolutionarily conserved molecules also present in humans. Here, focusing on antiviral immunity, we argue that immune genes not conserved in humans represent an unexplored resource for the discovery of new antiviral strategies. We review recent findings on the cGAS-STING pathway and explain how cyclic dinucleotides produced by cGAS-like receptors may be used to investigate the portfolio of antiviral genes in a broad range of species. This will set the stage for evo-immuno approaches, exploiting the investment in antiviral defences made by metazoans over hundreds million years of evolution.

Evolution to understand antiviral immunity: lessons from evo-devo.

As we have been reminded with the recent Covid-19 crisis, viruses represent a major threat for our societies. In humans, acute or chronic viral infections are associated with many life-threatening diseases, including cancer. Innate immunity is the first line of defence that operates in all animals and, in the case of vertebrates, precedes and orients the establishment of adaptive immunity. Host cells have evolved numerous innate defences against viral infections, but the control of viruses is complicated by the high mutation rates of most viral polymerases, which promote rapid virus evolution and adaptation to antiviral mechanisms. As a result, animal genomes have been shaped by continuous challenge from viruses and the repertoire of antiviral genes in any species reflects the cumulative effects of million years of evolutionary investment in innate immunity defenses[1,2]. At a time when we have been reminded that (i) we cannot predict what the next viral human pathogen will be and (ii) the risks of future zoonosis outbreaks are higher than ever, increasing the diversity of approaches to understand virus-host interaction should be a priority[3,4]. Studying these interactions in as many settings as possible will provide insight on a wide range of host restriction factors, opening the way to new applications in biomedicine.

The concept of evo-immuno –for evolutionary immunology– is inspired by the spectacular successes of the field of evolutionary developmental biology, or evo-devo, in the past 30 years. The evo-devo approach exploited genomics and genetics to gain insight on the developmental mechanisms underlying the evolution of morphological diversity in animals[5,6]. The crucial discovery that *homeobox* (*Hox*) genes control antero-posterior patterning in both flies and humans provided the first insight into a genetic toolkit shared for the development of morphologically very

different animals and paved the way for the evo-devo field[7,8]. A key aspect of the genetic theory for the evolution of animal morphology revealed by evo-devo and apparent in the hourglass scheme of Denis Duboule is that a conserved gene toolkit (e.g. *Hox* genes) responds to novelty and regulates novelty, hence providing adaptation to changing environments (**Fig. 1**)[7,8]. Indeed, the ontogenic trajectory is often influenced by environmental factors, and innovations in morphogenesis are generally selected if they improve adaptation of the animal to its environment or if they facilitate access to a new niche[9]. Besides embryonic development, the immune system is another facet of animal biology that is forged by interactions with the environment (e.g., the microbial world) and could benefit from evolutionary perspectives[10,11]. Of note, the model organism *Drosophila melanogaster*, with its extensive genetic and molecular resources, played a central role in evo-devo and other insects provided spectacular examples of emergence of striking morphological novelties from the ancestral conserved toolkit (e.g., refs[12–14]).

Evo-immuno to delve into the biodiversity of antiviral defence strategies in insects

Authors exploring comparative immunology have noted that, while signalling pathways tend to be conserved between species, the receptors sensing infection are less conserved and evolve rapidly, as do the effector molecules regulated by these pathways (see for example ref.[15–17] and Khimovitch & Bosch in this issue). Hence, innate immunity pathways exhibit an information bottleneck, akin to the bottleneck in gene expression corresponding to the establishment of the axis of the embryo during development. Although the evo-immuno concept does not entail an ontogeny aspect, it aims at exploiting central evolutionarily conserved regulatory nodes to gain insight

on original solutions to adapt to environmental challenges, following in this regard the path of evo-devo (**Fig. 1**). As argued elsewhere in this issue (see article by Hanson), all microbes have specific weaknesses, which can be exploited by host defense mechanisms and thus drive evolution of immune systems. Evo-immuno aims at identifying evolutionarily exploited weak spots in pathogens, which could point to innovative therapeutic strategies.

We propose to explore the concept of evo-immuno in insects, taking advantage of the *Drosophila* model, but also of the fantastic biodiversity of this class of animals with more than 1.2 million species known and well-established phylogenetic relationships[18]. Insects are present and play vital roles in all terrestrial ecosystems, where they are exposed to a broad range of pathogens, including viruses. Indeed, recent virome analysis have revealed that insects represent an impressive reservoir of viruses, including most major viral groups found in animals and plants (e.g. poxviruses, flaviviruses, alphaviruses, rhabdoviruses, picorna-like viruses, …)[19–21]. Furthermore, important human viruses are transmitted by hematophagous insect vectors such as *Aedes* mosquitoes[22]. Hence, characterizing antiviral immunity in insects may reveal original strategies of antiviral defences. Importantly, evo-immuno aims at understanding how a conserved gene toolkit produces a diversity of non-conserved responses, just as evo-devo allowed to understand how a conserved toolkit can build legs, wings, or fins at locations of the body. Hence, an important aspect of evo-immuno is that the study of non-conserved genes is as interesting as –or even, arguably, more interesting than– evolutionarily conserved ones. This represents an important paradigm shift as, until now, studies on insect immunity received much more support and publicity when they reported evolutionarily conserved mechanisms (e.g., Toll receptors)[23]. Yet, investigating non-

conserved genes holds much promise in the context of antiviral immunity. Restriction of a virus by a host factor will put pressure on the virus to adapt, often resulting in the emergence of resistant variants. This will in turn put pressure on the host to either modify the restriction factor to recover interaction with its viral target, or to find another solution to control the virus[24]. Because this continuous evolutionary arms race between two genetic entities antagonizing each other goes on permanently, the repertoire of antiviral factors is evolving rapidly[2,25]. Central to the concept of evo-immuno is the notion that this arms race goes on in parallel in all animals, such that each animal may find its own unique solution to counter a virus (**Fig. 2**). Thus, exploring the genomes of insects for species-specific innovations in antiviral immunity may reveal novel antiviral strategies.

Although antiviral immunity in insects has been a focus of attention in the past years, studies have focused on few species (flies, vector mosquitoes, honeybees) and specific viruses (e.g., ref.[26–29]). Broader investigations have been hampered by lack of information on the viruses infecting insects, which could be used to trigger antiviral immunity. The discovery of the important role played by the Stimulator of interferon genes (STING) pathway in the antiviral defence of *D. melanogaster* opens new perspectives[30]. Indeed, STING-dependent signalling can be activated *in vivo* by injection of cyclic dinucleotides[31–34]. This provides a powerful mean to trigger antiviral immunity in a range of insects, paving the way for evo-immuno (**Fig. 1**).

STING signaling participates in insect antiviral immunity

In the course of our work on induced-antiviral responses in *D. melanogaster*[35–38], we discovered that two components of the antibacterial immune deficiency (IMD)

pathway, the kinase IKKβ and the NF-κB factor Relish, rather than the pathway as a whole[30], participated in the control of infection by two picorna-like viruses, Drosophila C virus (DCV) and Cricket paralysis virus (CrPV). It is worth reminding here that the characterization of *Diedel*, a gene not conserved in mammals but strongly induced in response to viral infection in *D. melanogaster* and hijacked by members of several families of large insect DNA viruses, attracted our attention to the possible involvement of components of the IMD pathway in antiviral immunity[39]. This illustrates how investigating non-conserved genes may reveal important evolutionarily conserved facets of immunity. We further identified the orthologue of STING, dSTING, among the genes regulated by IKKβ in the context of viral infections and showed that dSTING was acting upstream of IKKβ and Relish to regulate expression of genes induced by viral infections[30] (**Fig. 3**). Similar results were obtained in the silkworm *Bombyx mori*[40]. Altogether, our findings pointed to the existence of a new pathway activated by viruses and controlling expression of *STING-regulated genes* to curb viral infection.

In mammals, STING is a key component of the cytosolic DNA sensing pathway[41,42]. It activates the transcription factor IRF3 through the kinase TBK1 to induce interferon (IFN) gene expression, but also regulates IKKβ and NF-κB through a less characterized mechanism[43–45]. STING acts as a signalling receptor for a second messenger, the cyclic dinucleotide 2′3′-cGAMP, which is produced by the enzyme cGAS upon sensing cytosolic DNA[41,42] (**Fig. 3**). STING can also be activated by other cyclic dinucleotides directly produced by bacteria (e.g., 3′3′-c-di-AMP, 3′3′-c-di-GMP and 3′3′-cGAMP)[46].

In *Drosophila*, we showed that injection of cyclic dinucleotides into flies leads to a dose-dependent induction of STING-regulated genes, in a dSTING- and Relish-

dependent manner. Of note, 2′3′-cGAMP was a more potent agonist of dSTING than 3′3′-connected cyclic dinucleotides of bacterial origin, suggesting that an enzyme producing 2′3′-cGAMP was present in insects[31]. This discovery led to an analysis of the transcriptome of 2′3′-cGAMP -injected flies, which revealed more than 400 genes stimulated at least 1.5-fold 6, 12 or 24h post injection. It is worth mentioning here that 2′3′-cGAMP-induced genes include components of the small interfering RNA (*Dicer-2*, *Argonaute 2*) and autophagy (*Ref(2)P*, encoding the homologue of p62) pathways, pointing to interactions between the STING pathway and other antiviral mechanisms[47–52]. Strikingly, co-injection of 2′3′-cGAMP with viruses reduced viral replication and improved the survival of wild-type flies, but not *dSTING* or *Relish* mutant flies. This effect was observed on 5 different viruses belonging to different families (*Dicistroviridae*, *Nodaviridae*, *Alphaviridae*, *Rhabdoviridae*, *Nudiviridae*), indicating that 2′3′-cGAMP triggers broad antiviral immunity in *D. melanogaster*[31]. Altogether, our results revealed that 2′3′-cGAMP triggers a dSTING/NF-κB-dependent antiviral transcriptional response.

cGLRs, an emerging family of pattern recognition receptors

Two cGAS-like receptors, cGRL1 and cGLR2, acting upstream of STING in *D. melanogaster* have recently been identified[33,53]. Although they synthesize cyclic dinucleotides, like cGAS, their characterization revealed intriguing differences with the mammalian enzyme (**Fig. 3**)[54]. For one, the activity of cGLR1 *in vitro* or in transfected mammalian cells depends on the presence of double stranded (ds)RNA, rather than DNA. The *in vitro* activity of recombinant cGLR2 from the species *D. bipectinata* and *D. pseudoananassae* is also enhanced most strongly in the presence of dsRNA[32]. Another notable difference with cGAS is that *Drosophila* cGLRs produce

193 at least three cyclic dinucleotides in addition to 2′3′-cGAMP, which can all activate
194 STING signalling *in vivo*, albeit with different efficiencies (2′3′-c-di-GMP>3′2′-
195 cGAMP>2′3′-c-di-AMP>2′3′-cGAMP)[32].

196 cGLRs represent an emerging family of pattern recognition receptors (PRRs)
197 present in all metazoan phyla and producing an array of cyclic di-purine and purine-
198 pyrimidine signals in response to binding DNA, RNA and probably also other
199 signals[55]. The biological significance of this diversity of cyclic dinucleotide products is
200 still unclear but could reflect the existence of alternative receptors for these
201 nucleotide signals. For example, the stony coral *Stylophora pistillata* encodes 42
202 cGLRs and 7 STING paralogs, which exhibit different affinity and selectivity for cyclic
203 dinucleotides: Sp-STING3 preferentially binds 3′3′-linked cyclic dinucleotides, while
204 Sp-STING5 is highly selective for 2′3′-cGAMP[55]. Other receptors for cyclic
205 dinucleotides exist in animals, e.g., RECON (reductase controlling NF-κB) in
206 mice[56,57]. Interestingly, flies synthesize an unusual cGAMP isomer, 3′2′-cGAMP,
207 which has so far only been identified in *Drosophila* and the bacteria *Asticcacaulis*
208 *sp.*[33,53,58,59]. This *Drosophila* innovation may have been driven by a family of viral
209 suppressors of cGAS-STING signalling, the Poxins[33]. Poxins were initially identified
210 in vaccinia virus, a member of the family *Poxviridae*, and function as 2′3′-cGAMP-
211 specific nucleases[60]. Strikingly, *poxin* homologs can be found in the genomes of
212 several large DNA viruses (baculoviruses, entomopoxviruses) infecting Lepidopteran
213 insects, but also in the genome of the moths and butterflies that host these viruses,
214 pointing to a likely insect origin for *poxin* genes[61]. Poxin efficiently cleaves 2′3′-
215 cGAMP but fails to cleave 3′2′-cGAMP, suggesting that the isomeric switch in
216 phosphodiester linkage specificity in *Drosophila* may have occurred to evade its
217 action[33]. It is worth noting here that genomic analysis of different poxvirus genera

indicates that Poxins are widely found in insect poxviruses and are present in some bat and rodent poxviruses but are missing in many other vertebrate poxviruses. This suggests that Poxin was acquired by horizontal transfer from insect viruses, possibly favoured by the insectivorous nature of bats and rodents[62]. Overall, these findings illustrate the relevance of taking a broad look at the repertoire of antiviral defences present in animals, rather than focusing on only a few species.

The cGLR-CDN-STING cassette in the animal toolkit of antiviral defences

It is now clear that the connection of the STING pathway to the regulation of interferon genes is a late addition to an ancestral pathway, which has been associated with the control of viral infections long before the onset of vertebrates. Indeed, the three key components of the pathway – cGAS-like enzymes, the cyclic oligonucleotides they produce and STING-related molecules – were all inherited from prokaryotes, where they function in the control of phage infections (reviewed in ref.[63]). Accordingly, genes encoding cGLRs and STING are present in the genomes of invertebrates, although they were lost in some species, such as worms or, within insects, mosquitoes. In addition, an array of distinct cyclic dinucleotide signals controling discrete STING signaling pathways has recently been identified in a set of invertebrates[55]. Although the exact biological function of cGLRs in most animals remains unknown, the function of cGAS/DncV-like nucleotidyltransferase (CD-NTase) enzymes in the control of highly divergent anti-phage defense signaling pathways in bacteria[64] ; the conserved function of cGAS and cGLRs in antiviral immunity in flies and mammals[54] ; and the fact that most cGLRs can be activated *in vitro* by nucleic acids[33,53,55,58] – a molecular pattern characteristic of viral infections – altogether strongly suggest that the cGLR/STING signalling axis belongs to an

ancestral toolkit associated with the control of viral infections[63]. Therefore, this pathway can be harnessed to gain insight on the diversity of induced antiviral defences in animals, much like the way *Hox* genes were used for evo-devo (**Fig. 1**).

Induction of antiviral gene expression can be achieved by analyzing the transcriptome of cells or animals after viral infection. However, it is difficult in these studies to distinguish between the stress reaction triggered by cell lysis or tissue damage and the immune response. The analysis is further complicated by the fact that cell infections are not synchronized when animals are used instead of cell lines, preventing the distinction between immediate early responses and late responses to the infection. Finally, viruses are notorious for hijacking cellular functions and suppressing host defence mechanisms. The image provided by these transcriptomic studies can therefore be imprecise. This caveat can be avoided by stimulating cells with molecules mimicking the molecular patterns sensed by PRRs to trigger antiviral immunity. However, this is not trivial in the case of viral infections, since the nucleic acid mimics known to trigger antiviral immunity have to be delivered into cells, to meet PRRs residing in the cytosol or the endosomes[65]. In this context, the ability to activate STING signalling by injection of cyclic dinucleotides provides a powerful shortcut to visualize the modifications of the transcriptome associated with induction of antiviral immunity, in *Drosophila* and also other invertebrates[31]. The emerging diversity of cyclic dinucleotide signals produced by cGLRs represents a caveat but should not be limiting. Indeed, although cGLRs from invertebrates can produce several cyclic dinucleotides, 2′3′-cGAMP is produced by cGLRs from (i) several insects (e.g., *Drosophila* flies[32,33,53], the beetle *Tribolium castaneum*[33], the thrip *Frankliniella occidentalis* and the flea *Ctenocephalides felis*[55]); (ii) the sea anemone *Nematostella vectensis*[50,66]; (iii) the coral *Pocillopora damicornis*[55] and (iv) the fresh-

water polyp *Hydra vulgaris*[55]. Furthermore, as described below, 2′3′-cGAMP can activate STING signaling in many species, including *Drosophila* flies. If necessary (e.g., lack of significant response to 2′3′-cGAMP), other cyclic dinucleotides may be used, as shown recently for the *Drosophila* species *D. serrata* and 2′3′-c-diGMP[32]. Indeed, approaches suitable for chemical synthesis of cyclic dinucleotides with any combination of nucleobases and their purification have been described[67].

CDN injection provides access to a repertoire of antiviral genes

Injection of 2′3′-cGAMP or other cyclic dinucleotides has now been reported to activate an extensive transcriptional program not only in *Drosophila*, but also the sea anemone *N. vectensis*, the coral *S. pistillata*, the Eastern oyster *Crassostrea virginica* and even the marine chanoflagellate *Monosiga brevicollis*, a free living unicellular and colonial eukaryote that is the closest living relative of metazoans[31,55,68,69]. Strikingly, comparison of the genes differentially expressed in response to CDN injection in these organisms reveals a common pool of immune genes – including transcription factors (e.g., NF-κB, IRF), PRRs (e.g., cGLRs; RIG-I-like receptors; Nucleotide binding domain, Leucine-rich repeats containing Receptors (NLRs)) or effectors (e.g., viperin, OAS, RNaseL) –, pointing to an ancestral set of immunity factors that have been conserved over more than 500 million years of evolution. A number of these genes (e.g., viperin, Argonaute or cGLRs and STING themselves, as mentioned above) have been inherited from the arsenal of antiphage defences in prokaryotes[70]. The function of the mammalian orthologues of several of these factors has already been characterized[71]. However, besides this evolutionarily ancient and conserved program, many non-conserved genes are also induced in all species. These genes are evidence of divergent evolution in some organisms to adapt to the

293 threat of viruses, which may have resulted in unique strategies to restrict viral
294 replication (**Fig. 2**). Examples of these clade-specific innovations include the onset of
295 the IFN family of cytokines in vertebrates, or the fly-specific gene *pastrel*, which
296 encodes a potent restriction factor for picorna-like viruses and whose expression is
297 induced by 2′3′-cGAMP in all ten *Drosophila* species analysed[72,73] (Hédelin, Thiébaut
298 *et al*, manuscript in preparation). In invertebrates, it appears that, at least in some
299 species (e.g., *N. vectensis*, *M. brevicollis*), the STING pathway regulates expression
300 of antibacterial genes, in addition to antiviral genes, which may represent an early
301 innovation in the animal lineage of multicellular eukaryotes to connect the pathway to
302 infectious agents beyond viruses[68,69].

303 A few years ago, Palmarini, Wilson and colleagues investigated in a pioneer
304 study the “type-I interferomes” of fibroblasts from ten species of vertebrates and
305 identified a conserved core of 62 IFN-stimulated genes (ISG), thus highlighting the
306 ancestral functions of the IFN response[74]. Notably, this study also revealed that each
307 animal possessed ISGs unique to their species or their phylogenetic lineage. This
308 attests to the constant expansion of ISGs in vertebrates, most likely driven by the
309 constant arms race between host and viruses[2]. Of note, many ISGs are also induced
310 directly upon sensing viral infection by PRRs and predate the evolution of interferons,
311 such that they have homologs in invertebrates and even in procaryotes[70,75,76]. The
312 possibility to investigate the “STINGome” of invertebrates using the cyclic
313 dinucleotide injection assay now provide the opportunity to investigate evolution of
314 antiviral responses in these animals as well, which offer access to a much broader
315 biodiversity. In particular, with the tools now at hand, insects represent powerful
316 models to identify new restriction factors against families of viruses with broad host
317 tropisms, such as *Picornaviridae*, *Poxviridae*, *Flaviviridae*, *Togaviridae*.

Concluding remarks

It is now acknowledged that most animals control viral infection through the induction of dedicated gene expression programs. Yet only a fraction of mammalian ISGs have been functionally characterized and recent studies reveal that a substantial number of genes induced by viral infection are not conserved between animals[77]. Furthermore, recent insight on the emerging role of the evolutionarily conserved cGLR/STING pathway positions it as a central component of the toolkit used to fight viral infections in animals beyond vertebrates. Induction of STING-dependent antiviral immunity upon cyclic dinucleotide injection allows rapid identification in different species of large sets of genes regulated in context of viral infections, paving the way for the exploration of the putative antiviral functions of non-conserved genes. It is noteworthy that, although we focus here on viruses, the concept of evo-immuno also applies to other infections. Indeed, pioneering studies in innate immunity revealed that, although Toll signalling to NF-κB is conserved between insects and mammals, Toll functions as a cytokine receptor in *Drosophila*, unlike Toll-like receptors, which function as PRRs[78–81]. Furthermore, recent studies indicate that the antimicrobial peptides regulated by NF-κB in different *Drosophila* species are subjected to diversification and specialization driven by differences in the ecologies of the flies[82].

In summary, the breakthrough discovery that broad antiviral immunity can be triggered in *Drosophila* and other invertebrates by injection of CDNs provides a rapid and reliable way to access to the repertoire of antiviral genes[31,33,55,58,68,69]. The next step will be to exploit these lists of genes to identify novel restriction factors. This will be challenging, but large-scale biochemical analysis aided by structural predictions[83]

and evolution-guided pipelines[84] to select non conserved candidate genes with interesting features, represents a powerful strategy for discovery. Mechanistic studies on the molecular function of these non-conserved genes may lead to the discovery of potentially unique strategies of antiviral defence, revealing discrete weak spots in the targeted viruses. These could be exploited to develop innovative antiviral therapies. As we will continue to be confronted with emerging viruses that ignore phylogeny when they jump into new hosts, it is time to extend the search for antiviral restriction mechanisms beyond humans or mammals and to acknowledge that what is not conserved can be as important as what is conserved, as superbly illustrated by the evo-devo field.

Acknowledgements and funding

We thank Joao Marques and members of our research groups for stimulating discussions. J.L.I. acknowledges financial support from CNRS, the Hoffmann Infinitus Program and grants from ANR (ANR-11-EQPX-0022, ANR-22-CE15-0019), IdEx Unistra (ANR-10-IDEX-0002), SFRI-STRAT'US (ANR-20-SFRI0012) and EUR IMCBio (ANR-17-EURE-0023). H.C. was supported by the Natural Science Foundation (32000662), Guangdong Provincial Science Fund for Distinguished Young Scholars (2023B1515020098), Guangdong Provincial Young Scholars academic exchange program (2022A0505030018), Youth Talent Support Programme of Guangdong Provincial Association for Science and Technology (SKXRC202229), Science and Technology Program of Guangzhou (202102020090).

References

1. Goodin, M.M., Hatfull, G.F., and Malik, H.S. (2016). A Diversified Portfolio. Annu Rev Virol *3*, vi–viii. 10.1146/annurev-vi-3-100316-100011.
2. Tenthorey, J.L., Emerman, M., and Malik, H.S. (2022). Evolutionary Landscapes of Host-Virus Arms Races. Annu Rev Immunol. 10.1146/annurev-immunol-072621-084422.
3. Dobson, A.P., Pimm, S.L., Hannah, L., Kaufman, L., Ahumada, J.A., Ando, A.W., Bernstein, A., Busch, J., Daszak, P., Engelmann, J., et al. (2020). Ecology and economics for pandemic prevention. Science *369*, 379–381. 10.1126/science.abc3189.
4. Gibb, R., Redding, D.W., Chin, K.Q., Donnelly, C.A., Blackburn, T.M., Newbold, T., and Jones, K.E. (2020). Zoonotic host diversity increases in human-dominated ecosystems. Nature *584*, 398–402. 10.1038/s41586-020-2562-8.
5. Carroll, S.B. (2008). Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell *134*, 25–36. 10.1016/j.cell.2008.06.030.
6. De Robertis, E.M. (2008). Evo-devo: variations on ancestral themes. Cell *132*, 185–195. 10.1016/j.cell.2008.01.003.
7. Duboule, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. Development *1994*, 135–142. 10.1242/dev.1994.Supplement.135.
8. Duboule, D. (2022). The (unusual) heuristic value of Hox gene clusters; a matter of time? Developmental Biology *484*, 75–87. 10.1016/j.ydbio.2022.02.007.
9. Mallarino, R., and Abzhanov, A. (2012). Paths less traveled: evo-devo approaches to investigating animal morphological evolution. Annu. Rev. Cell Dev. Biol. *28*, 743–763. 10.1146/annurev-cellbio-101011-155732.
10. Madhani, H.D. (2013). The Frustrated Gene: Origins of Eukaryotic Gene Expression. Cell *155*, 744–749. 10.1016/j.cell.2013.10.003.
11. Enard, D., Cai, L., Gwennap, C., and Petrov, D.A. (2016). Viruses are a dominant driver of protein adaptation in mammals. eLife *5*, e12469. 10.7554/eLife.12469.
12. Arnoult, L., Su, K.F.Y., Manoel, D., Minervino, C., Magriña, J., Gompel, N., and Prud'homme, B. (2013). Emergence and diversification of fly pigmentation through evolution of a gene regulatory module. Science *339*, 1423–1426. 10.1126/science.1233749.
13. Prud'homme, B., Minervino, C., Hocine, M., Cande, J.D., Aouane, A., Dufour, H.D., Kassner, V.A., and Gompel, N. (2011). Body plan innovation in treehoppers through the evolution of an extra wing-like appendage. Nature *473*, 83–86. 10.1038/nature09977.
14. Santos, M.E., Le Bouquin, A., Crumière, A.J.J., and Khila, A. (2017). Taxon-restricted genes at the origin of a novel trait allowing access to a new environment. Science *358*, 386–390. 10.1126/science.aan2748.
15. Tassia, M.G., Whelan, N.V., and Halanych, K.M. (2017). Toll-like receptor pathway evolution in deuterostomes. Proceedings of the National Academy of Sciences *114*, 7055–7060. 10.1073/pnas.1617722114.
16. Mosallanejad, K., Kennedy, S.N., Bahleda, K.M., Slavik, K.M., Zhou, W., Govande, A.A., Hancks, D.C., Kranzusch, P.J., and Kagan, J.C. (2023). Species-specific self-DNA detection mechanisms by mammalian cyclic GMP-AMP synthases.

Science Immunology *8*, eabp9765. 10.1126/sciimmunol.abp9765.
17. Leulier, F., and Lemaitre, B. (2008). Toll-like receptors — taking an evolutionary approach. Nat Rev Genet *9*, 165–178. 10.1038/nrg2303.
18. Jactel, H., Imler, J.-L., Lambrechts, L., Failloux, A.-B., Lebreton, J.D., Maho, Y.L., Duplessy, J.-C., Cossart, P., and Grandcolas, P. (2021). Insect decline: immediate action is needed. C R Biol *343*, 267–293. 10.5802/crbiol.37.
19. Shi, M., Lin, X.-D., Tian, J.-H., Chen, L.-J., Chen, X., Li, C.-X., Qin, X.-C., Li, J., Cao, J.-P., Eden, J.-S., et al. (2016). Redefining the invertebrate RNA virosphere. Nature *540*, 539–543. 10.1038/nature20167.
20. Li, C.-X., Shi, M., Tian, J.-H., Lin, X.-D., Kang, Y.-J., Chen, L.-J., Qin, X.-C., Xu, J., Holmes, E.C., and Zhang, Y.-Z. (2015). Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Elife *4*, e05378. 10.7554/eLife.05378.
21. Aguiar, E.R.G.R., Olmo, R.P., Paro, S., Ferreira, F.V., de Faria, I.J. da S., Todjro, Y.M.H., Lobo, F.P., Kroon, E.G., Meignin, C., Gatherer, D., et al. (2015). Sequence-independent characterization of viruses based on the pattern of viral small RNAs produced by the host. Nucleic Acids Res. *43*, 6191–6206. 10.1093/nar/gkv587.
22. Shaw, W.R., and Catteruccia, F. (2019). Vector biology meets disease control: using basic research to fight vector-borne diseases. Nat Microbiol *4*, 20–34. 10.1038/s41564-018-0214-7.
23. Hoffmann, J.A. (2003). The immune response of Drosophila. Nature *426*, 33–38. 10.1038/nature02021.
24. Daugherty, M.D., and Malik, H.S. (2012). Rules of engagement: molecular insights from host-virus arms races. Annu. Rev. Genet. *46*, 677–700. 10.1146/annurev-genet-110711-155522.
25. Duggal, N.K., and Emerman, M. (2012). Evolutionary conflicts between viruses and restriction factors shape immunity. Nat. Rev. Immunol. *12*, 687–695. 10.1038/nri3295.
26. Palmer, W.H., Varghese, F.S., and van Rij, R.P. (2018). Natural Variation in Resistance to Virus Infection in Dipteran Insects. Viruses *10*. 10.3390/v10030118.
27. Niu, J., Meeus, I., De Coninck, D.I., Deforce, D., Etebari, K., Asgari, S., and Smagghe, G. (2017). Infections of virulent and avirulent viruses differentially influenced the expression of dicer-1, ago-1, and microRNAs in Bombus terrestris. Sci Rep *7*, 45620. 10.1038/srep45620.
28. Bonning, B.C., and Saleh, M.-C. (2021). The Interplay Between Viruses and RNAi Pathways in Insects. Annu Rev Entomol *66*, 61–79. 10.1146/annurev-ento-033020-090410.
29. Tikhe, C.V., and Dimopoulos, G. (2021). Mosquito antiviral immune pathways. Developmental & Comparative Immunology *116*, 103964. 10.1016/j.dci.2020.103964.
30. Goto, A., Okado, K., Martins, N., Cai, H., Barbier, V., Lamiable, O., Troxler, L., Santiago, E., Kuhn, L., Paik, D., et al. (2018). The Kinase IKKβ Regulates a STING- and NF-κB-Dependent Antiviral Response Pathway in Drosophila. Immunity *49*, 225-234.e4. 10.1016/j.immuni.2018.07.013.
31. Cai, H., Holleufer, A., Simonsen, B., Schneider, J., Lemoine, A., Gad, H.H., Huang, J., Huang, J., Chen, D., Peng, T., et al. (2020). 2'3'-cGAMP triggers a STING- and NF-κB-dependent broad antiviral response in Drosophila. Sci Signal *13*. 10.1126/scisignal.abc4537.
32. Cai, H., Li, L., Slavik, K.M., Huang, J., Yin, T., Ai, X., Hédelin, L., Haas, G., Xiang, Z., Yang, Y., et al. (2023). The virus-induced cyclic dinucleotide 2′3′-c-di-GMP mediates STING-dependent antiviral immunity in Drosophila. Immunity *56*, 1991-

2005.e9. 10.1016/j.immuni.2023.08.006.
33. Slavik, K.M., Morehouse, B.R., Ragucci, A.E., Zhou, W., Ai, X., Chen, Y., Li, L., Wei, Z., Bähre, H., König, M., et al. (2021). cGAS-like receptors sense RNA and control 3'2'-cGAMP signalling in Drosophila. Nature *597*, 109–113. 10.1038/s41586-021-03743-5.
34. Segrist, E., Dittmar, M., Gold, B., and Cherry, S. (2021). Orally acquired cyclic dinucleotides drive dSTING-dependent antiviral immunity in enterocytes. Cell Reports *37*, 110150. 10.1016/j.celrep.2021.110150.
35. Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J.A., and Imler, J.-L. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nat. Immunol. *6*, 946–953. 10.1038/ni1237.
36. Deddouche, S., Matt, N., Budd, A., Mueller, S., Kemp, C., Galiana-Arnoux, D., Dostert, C., Antoniewski, C., Hoffmann, J.A., and Imler, J.-L. (2008). The DExD/H-box helicase Dicer-2 mediates the induction of antiviral activity in drosophila. Nat. Immunol. *9*, 1425–1432. 10.1038/ni.1664.
37. Kemp, C., Mueller, S., Goto, A., Barbier, V., Paro, S., Bonnay, F., Dostert, C., Troxler, L., Hetru, C., Meignin, C., et al. (2013). Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in Drosophila. J. Immunol. *190*, 650–658. 10.4049/jimmunol.1102486.
38. Lamiable, O., Arnold, J., de Faria, I.J. da S., Olmo, R.P., Bergami, F., Meignin, C., Hoffmann, J.A., Marques, J.T., and Imler, J.-L. (2016). Analysis of the Contribution of Hemocytes and Autophagy to Drosophila Antiviral Immunity. J. Virol. *90*, 5415–5426. 10.1128/JVI.00238-16.
39. Lamiable, O., Kellenberger, C., Kemp, C., Troxler, L., Pelte, N., Boutros, M., Marques, J.T., Daeffler, L., Hoffmann, J.A., Roussel, A., et al. (2016). Cytokine Diedel and a viral homologue suppress the IMD pathway in Drosophila. Proc. Natl. Acad. Sci. U.S.A. *113*, 698–703. 10.1073/pnas.1516122113.
40. Hua, X., Li, B., Song, L., Hu, C., Li, X., Wang, D., Xiong, Y., Zhao, P., He, H., Xia, Q., et al. (2018). Stimulator of interferon genes (STING) provides insect antiviral immunity by promoting Dredd caspase-mediated NF-κB activation. J. Biol. Chem. *293*, 11878–11890. 10.1074/jbc.RA117.000194.
41. Ablasser, A., and Chen, Z.J. (2019). cGAS in action: Expanding roles in immunity and inflammation. Science *363*. 10.1126/science.aat8657.
42. Zhang, X., Bai, X.-C., and Chen, Z.J. (2020). Structures and Mechanisms in the cGAS-STING Innate Immunity Pathway. Immunity *53*, 43–53. 10.1016/j.immuni.2020.05.013.
43. Yamashiro, L.H., Wilson, S.C., Morrison, H.M., Karalis, V., Chung, J.-Y.J., Chen, K.J., Bateup, H.S., Szpara, M.L., Lee, A.Y., Cox, J.S., et al. (2020). Interferon-independent STING signaling promotes resistance to HSV-1 in vivo. Nat Commun *11*, 3382. 10.1038/s41467-020-17156-x.
44. Cerboni, S., Jeremiah, N., Gentili, M., Gehrmann, U., Conrad, C., Stolzenberg, M.-C., Picard, C., Neven, B., Fischer, A., Amigorena, S., et al. (2017). Intrinsic antiproliferative activity of the innate sensor STING in T lymphocytes. J. Exp. Med. *214*, 1769–1785. 10.1084/jem.20161674.
45. Wu, J., Dobbs, N., Yang, K., and Yan, N. (2020). Interferon-Independent Activities of Mammalian STING Mediate Antiviral Response and Tumor Immune Evasion. Immunity *53*, 115-126.e5. 10.1016/j.immuni.2020.06.009.
46. Burdette, D.L., Monroe, K.M., Sotelo-Troha, K., Iwig, J.S., Eckert, B., Hyodo, M., Hayakawa, Y., and Vance, R.E. (2011). STING is a direct innate immune sensor

of cyclic di-GMP. Nature *478*, 515–518. 10.1038/nature10429.
47. Wang, X.-H., Aliyari, R., Li, W.-X., Li, H.-W., Kim, K., Carthew, R., Atkinson, P., and Ding, S.-W. (2006). RNA interference directs innate immunity against viruses in adult Drosophila. Science *312*, 452–454. 10.1126/science.1125694.
48. Galiana-Arnoux, D., Dostert, C., Schneemann, A., Hoffmann, J.A., and Imler, J.-L. (2006). Essential function in vivo for Dicer-2 in host defense against RNA viruses in drosophila. Nat. Immunol. *7*, 590–597. 10.1038/ni1335.
49. van Rij, R.P., Saleh, M.-C., Berry, B., Foo, C., Houk, A., Antoniewski, C., and Andino, R. (2006). The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in Drosophila melanogaster. Genes Dev. *20*, 2985–2995. 10.1101/gad.1482006.
50. Gui, X., Yang, H., Li, T., Tan, X., Shi, P., Li, M., Du, F., and Chen, Z.J. (2019). Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. Nature *567*, 262–266. 10.1038/s41586-019-1006-9.
51. Shelly, S., Lukinova, N., Bambina, S., Berman, A., and Cherry, S. (2009). Autophagy is an essential component of Drosophila immunity against vesicular stomatitis virus. Immunity *30*, 588–598. 10.1016/j.immuni.2009.02.009.
52. Liu, Y., Gordesky-Gold, B., Leney-Greene, M., Weinbren, N.L., Tudor, M., and Cherry, S. (2018). Inflammation-Induced, STING-Dependent Autophagy Restricts Zika Virus Infection in the Drosophila Brain. Cell Host Microbe *24*, 57-68.e3. 10.1016/j.chom.2018.05.022.
53. Holleufer, A., Winther, K.G., Gad, H.H., Ai, X., Chen, Y., Li, L., Wei, Z., Deng, H., Liu, J., Frederiksen, N.A., et al. (2021). Two cGAS-like receptors induce antiviral immunity in Drosophila. Nature *597*, 114–118. 10.1038/s41586-021-03800-z.
54. Cai, H., Meignin, C., and Imler, J.-L. (2022). cGAS-like receptor-mediated immunity: the insect perspective. Current Opinion in Immunology *74*, 183–189. 10.1016/j.coi.2022.01.005.
55. Li, Y., Slavik, K.M., Toyoda, H.C., Morehouse, B.R., Mann, C.C. de O., Elek, A., Levy, S., Wang, Z., Mears, K.S., Liu, J., et al. (2023). cGLRs are a diverse family of pattern recognition receptors in innate immunity. Cell *186*, 3261-3276.e20. 10.1016/j.cell.2023.05.038.
56. McFarland, A.P., Luo, S., Ahmed-Qadri, F., Zuck, M., Thayer, E.F., Goo, Y.A., Hybiske, K., Tong, L., and Woodward, J.J. (2017). Sensing of Bacterial Cyclic Dinucleotides by the Oxidoreductase RECON Promotes NF-κB Activation and Shapes a Proinflammatory Antibacterial State. Immunity *46*, 433–445. 10.1016/j.immuni.2017.02.014.
57. Whiteley, A.T., Eaglesham, J.B., de Oliveira Mann, C.C., Morehouse, B.R., Lowey, B., Nieminen, E.A., Danilchanka, O., King, D.S., Lee, A.S.Y., Mekalanos, J.J., et al. (2019). Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. Nature *567*, 194–199. 10.1038/s41586-019-0953-5.
58. Cai, H., Li, L., Slavik, K., Huang, J., Yin, T., Hédelin, L., Xiang, Z., Yang, Y., Li, X., Chen, Y., et al. (2023). A novel virus-induced cyclic dinucleotide, 2′3′-c-di-GMP, mediates STING-dependent antiviral immunity in Drosophila. 2023.05.08.539652. 10.1101/2023.05.08.539652.
59. Fatma, S., Chakravarti, A., Zeng, X., and Huang, R.H. (2021). Molecular mechanisms of the CdnG-Cap5 antiphage defense system employing 3′,2′-cGAMP as the second messenger. Nat Commun *12*, 6381. 10.1038/s41467-021-26738-2.
60. Eaglesham, J.B., Pan, Y., Kupper, T.S., and Kranzusch, P.J. (2019). Viral and metazoan poxins are cGAMP-specific nucleases that restrict cGAS-STING signalling. Nature *566*, 259–263. 10.1038/s41586-019-0928-6.

61. Eaglesham, J.B., McCarty, K.L., and Kranzusch, P.J. (2020). Structures of diverse poxin cGAMP nucleases reveal a widespread role for cGAS-STING evasion in host-pathogen conflict. Elife *9*. 10.7554/eLife.59753.
62. Maluquer de Motes, C. (2021). Poxvirus cGAMP nucleases: Clues and mysteries from a stolen gene. PLoS Pathog *17*, e1009372. 10.1371/journal.ppat.1009372.
63. Slavik, K.M., and Kranzusch, P.J. (2023). CBASS to cGAS-STING: The Origins and Mechanisms of Nucleotide Second Messenger Immune Signaling. Annu Rev Virol. 10.1146/annurev-virology-111821-115636.
64. Duncan-Lowey, B., and Kranzusch, P.J. (2022). CBASS phage defense and evolution of antiviral nucleotide signaling. Current Opinion in Immunology *74*, 156–163. 10.1016/j.coi.2022.01.002.
65. de Oliveira Mann, C.C., and Hornung, V. (2021). Molecular mechanisms of nonself nucleic acid recognition by the innate immune system. European Journal of Immunology *51*, 1897–1910. 10.1002/eji.202049116.
66. Kranzusch, P.J., Wilson, S.C., Lee, A.S.Y., Berger, J.M., Doudna, J.A., and Vance, R.E. (2015). Ancient Origin of cGAS-STING Reveals Mechanism of Universal 2',3' cGAMP Signaling. Mol. Cell *59*, 891–903. 10.1016/j.molcel.2015.07.022.
67. Wang, C., Hao, M., Qi, Q., Chen, Y., and Hartig, J.S. (2019). Chapter Three - Chemical synthesis, purification, and characterization of 3′-5′-linked canonical cyclic dinucleotides (CDNs). In Methods in Enzymology DNA Sensors and Inflammasomes., J. Sohn, ed. (Academic Press), pp. 41–59. 10.1016/bs.mie.2019.04.022.
68. Woznica, A., Kumar, A., Sturge, C.R., Xing, C., King, N., and Pfeiffer, J.K. (2021). STING mediates immune responses in the closest living relatives of animals. eLife *10*, e70436. 10.7554/eLife.70436.
69. Margolis, S.R., Dietzen, P.A., Hayes, B.M., Wilson, S.C., Remick, B.C., Chou, S., and Vance, R.E. (2021). The cyclic dinucleotide 2′3′-cGAMP induces a broad antibacterial and antiviral response in the sea anemone Nematostella vectensis. PNAS *118*. 10.1073/pnas.2109022118.
70. Wein, T., and Sorek, R. (2022). Bacterial origins of human cell-autonomous innate immune mechanisms. Nat Rev Immunol *22*, 629–638. 10.1038/s41577-022-00705-4.
71. Schoggins, J.W. (2019). Interferon-Stimulated Genes: What Do They All Do? Annu Rev Virol *6*, 567–584. 10.1146/annurev-virology-092818-015756.
72. Magwire, M.M., Fabian, D.K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., and Jiggins, F.M. (2012). Genome-wide association studies reveal a simple genetic basis of resistance to naturally coevolving viruses in Drosophila melanogaster. PLoS Genet. *8*, e1003057. 10.1371/journal.pgen.1003057.
73. Martins, N.E., Faria, V.G., Nolte, V., Schlötterer, C., Teixeira, L., Sucena, É., and Magalhães, S. (2014). Host adaptation to viruses relies on few genes with different cross-resistance properties. Proc. Natl. Acad. Sci. U.S.A. *111*, 5938–5943. 10.1073/pnas.1400378111.
74. Shaw, A.E., Hughes, J., Gu, Q., Behdenna, A., Singer, J.B., Dennis, T., Orton, R.J., Varela, M., Gifford, R.J., Wilson, S.J., et al. (2017). Fundamental properties of the mammalian innate immune system revealed by multispecies comparison of type I interferon responses. PLoS Biol. *15*, e2004086. 10.1371/journal.pbio.2004086.
75. Sarkar, S.N., and Sen, G.C. (2004). Novel functions of proteins encoded by viral stress-inducible genes. Pharmacology & Therapeutics *103*, 245–259. 10.1016/j.pharmthera.2004.07.007.

76. Lafont, M., Vergnes, A., Vidal-Dupiol, J., de Lorgeril, J., Gueguen, Y., Haffner, P., Petton, B., Chaparro, C., Barrachina, C., Destoumieux-Garzon, D., et al. (2020). A Sustained Immune Response Supports Long-Term Antiviral Immune Priming in the Pacific Oyster, Crassostrea gigas. mBio *11*. 10.1128/mBio.02777-19.
77. McDougal, M.B., Boys, I.N., De La Cruz-Rivera, P., and Schoggins, J.W. (2022). Evolution of the interferon response: lessons from ISGs of diverse mammals. Current Opinion in Virology *53*, 101202. 10.1016/j.coviro.2022.101202.
78. Morisato, D., and Anderson, K.V. (1994). The spätzle gene encodes a component of the extracellular signaling pathway establishing the dorsal-ventral pattern of the Drosophila embryo. Cell *76*, 677–688. 10.1016/0092-8674(94)90507-X.
79. Schneider, D.S., Jin, Y., Morisato, D., and Anderson, K.V. (1994). A processed form of the Spätzle protein defines dorsal-ventral polarity in the Drosophila embryo. Development *120*, 1243–1250. 10.1242/dev.120.5.1243.
80. Levashina, E.A. (1999). Constitutive Activation of Toll-Mediated Antifungal Defense in Serpin-Deficient Drosophila. Science *285*, 1917–1919. 10.1126/science.285.5435.1917.
81. Weber, A.N.R., Tauszig-Delamasure, S., Hoffmann, J.A., Lelièvre, E., Gascan, H., Ray, K.P., Morse, M.A., Imler, J.-L., and Gay, N.J. (2003). Binding of the Drosophila cytokine Spätzle to Toll is direct and establishes signaling. Nat. Immunol. *4*, 794–800. 10.1038/ni955.
82. Hanson, M.A., Grollmus, L., and Lemaitre, B. (2023). Ecology-relevant bacteria drive the evolution of host antimicrobial peptides in Drosophila. Science *381*, eadg5725. 10.1126/science.adg5725.
83. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature *596*, 583–589. 10.1038/s41586-021-03819-2.
84. Picard, L., Ganivet, Q., Allatif, O., Cimarelli, A., Guéguen, L., and Etienne, L. (2020). DGINN, an automated and highly-flexible pipeline for the detection of genetic innovations on protein-coding genes. Nucleic Acids Research *48*, e103. 10.1093/nar/gkaa680.

Figure legends

Fig. 1. Parallel between evo-devo and evo-immuno.

The STING pathway can be used to probe original antiviral strategies, much like homeobox genes were used to decipher evolution of morphology traits in animals.

Fig. 2. Evolutionary arms race between hosts and viruses.

When confronted to rapidly evolving viruses that escape host restriction factors, host adapts either by modifying the restriction factor (deep blue) or by finding another target in the virus. As a result, each animal may exhibit lineage-specific antiviral restriction factors. Modified from ref.[24].

Fig. 3. Evolutionary conservation of the cGLR-CDN-STING cassette.

Enzymes of the CD-NTase family (cGAS, cGLRs, CdnE), the cyclic oligonucleotides the produce and STING related molecules participate in the control of phage or viral infections in bacteria and animals, from invertebrates to mammals. The mechanism activating CD-NTase enzymes in prokaryotes in response to phage infections is still unknown. Note that as predicted in the hourglass model shown in Fig. 1, cGLRs and cGAS are not activated by the same type of nucleic acids and regulate overlapping but distinct sets of genes (e.g., no interferons in invertebrates).

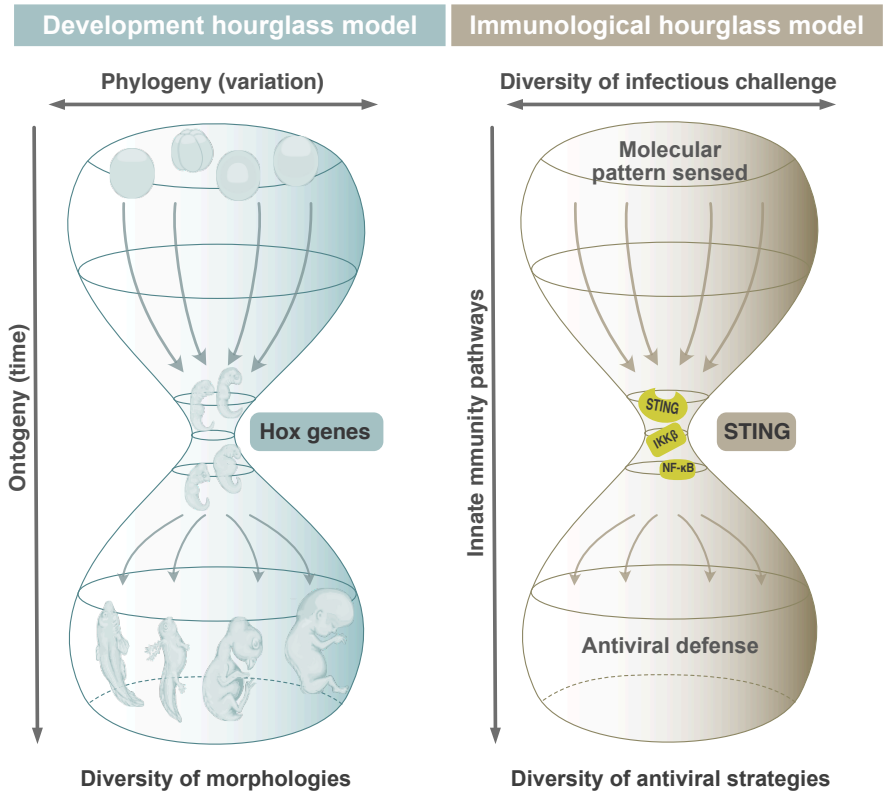

Figure 1: Parallel between evo-devo and evo-immuno. The STING pathway can be used to probe original antiviral strategies, much like homeobox genes were instrumental to decipher evolution of morphology in animals.

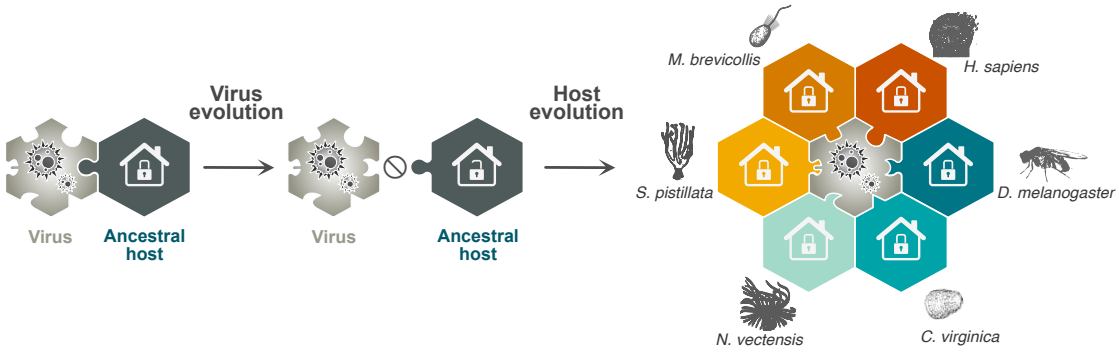

Figure 2: Evolutionary arms race between hosts and viruses. When confronted to rapidly evolving viruses that escape host restriction factors, hosts adapt by modifying the restriction factor (deep blue) or by finding another target in the virus. As a result, each animal may exhibit lineage-specific restriction factors. Modified from ref. 18.

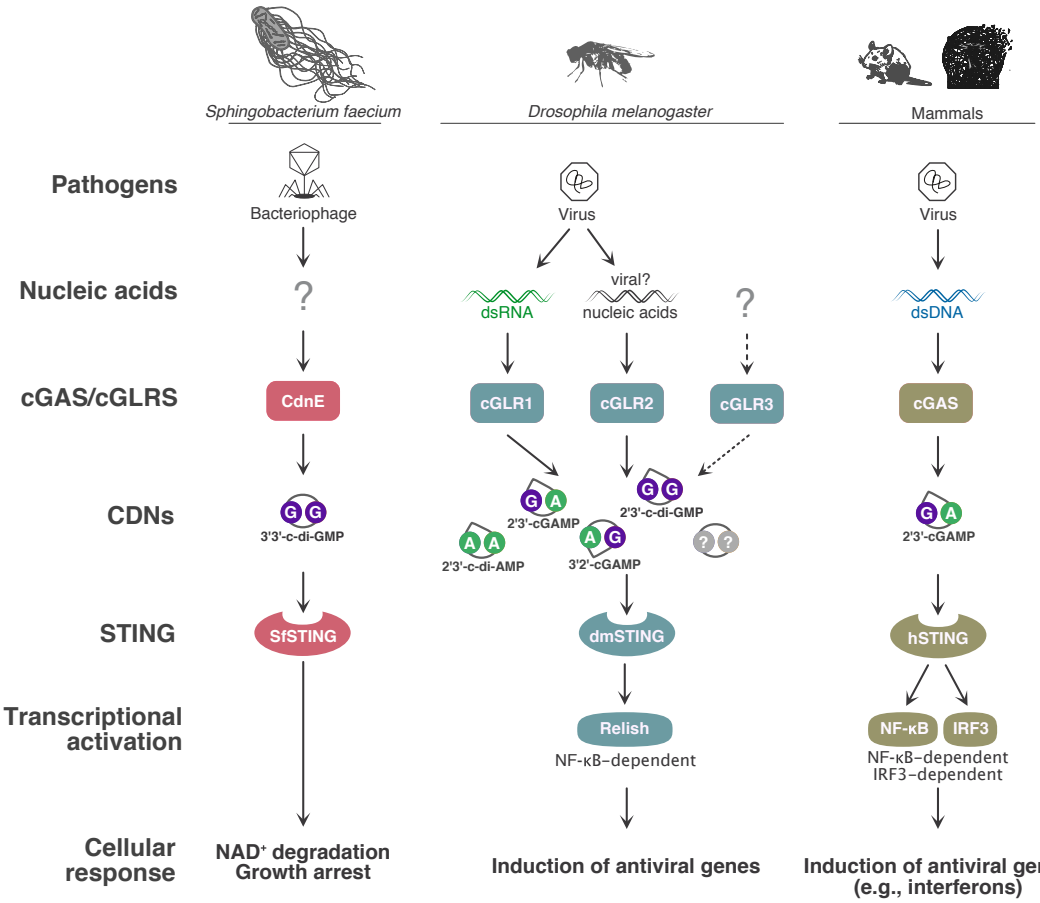

Figure 3: Evolutionary conservation of the cGLR-CDN-STING cassette. Enzymes of the CD-NTase family (cGAS, cGLRs, CdnE), the cyclic oligonucleotides they produce and STING related molecules participate in the control of phage or viral infections in bacteria and animals, from invertebrates to mammals. The mechanism activating CD-NTase enzymes in prokaryotes in response to phage infections is still unknown. Note that as predicted in the hourglass model shown in Fig. 1, cGLRs and cGAS are not activated by the same type of nucleic acids and regulate overlapping but distinct sets of genes (e.g., no interferons in invertebrates).