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Current Opinion in Insect Science

Saliva of hematophagous insects: a multifaceted toolkit

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Transcriptomic, proteomic and genomic studies significantly improved our understanding of the complexity of blood feeding insect saliva providing unparalleled evolutionary insights. Salivary genes appeared to be under strong selective pressure with gene duplication and functional diversification being a powerful driver in the evolution of novel salivary genes/functions. The first insect salivary proteins responsible for complement inhibition were identified and a widespread mechanism of action shared by unrelated salivary protein families was recognized and named kratagonism. microRNAs were for the first time described in the saliva of a few blood feeding arthropods raising intriguing questions on their possible contribution to vertebrate host manipulation and pathogen transmission and further emphasizing how much we still have to learn on blood feeding insect saliva.

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Current Opinion in Insect Science 2018, **29**:xx–yy
This review comes from a themed issue on **Molecular physiology**
Edited by **Pedro L Oliveira** and **Fernando G Noriega**

https://doi.org/10.1016/j.cois.2018.07.012

Introduction

The ability to use blood as food source conferred to hematophagous insects a considerable reproductive advantage but also involved the evolution of complex morphological, physiological and behavioral adaptations to allow insects to find suitable hosts, pierce their skin and then suck and digest blood [1,2]. The first blood feeding insect (BFI) appeared most likely ~200–150 million years ago in the late Jurassic-early Cretaceous [3]. However, hematophagy evolved independently several times: at least 5 times at the order level (in Diptera, Hemiptera, Lepidoptera, Phthiraptera and Siphonaptera) and possibly independently 3 times within Hemiptera and 10 times within Diptera, which include mosquitoes, sand flies, tsetse flies, black flies, stable flies and biting midges [4]. This convergent evolutionary nature of hematophagy resulted in the appearance of variegated solutions to common problems connected to this style of life, with saliva being probably the most striking example of this heterogeneity [5].

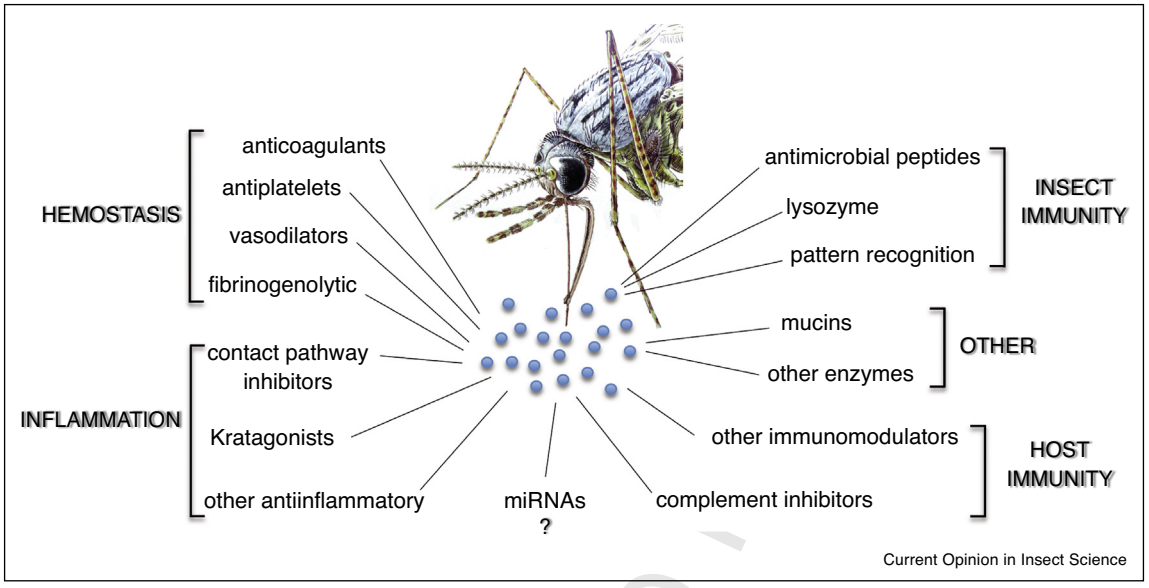
Saliva is known to help hematophagous insects to efficiently get their blood meals by interfering with vertebrate hemostasis, inflammation and immunity. Perhaps because of the need to counterbalance these complex and redundant host responses, BFIs evolved a salivary cocktail of similar complexity and redundancy carrying several dozen of bioactive compounds [6]. Salivary proteins directly affecting platelet activity and aggregation, coagulation cascade and vasodilation are certainly among the best characterized and provide several examples of convergent evolution [6,7••]. However, a large variety of other activities more or less directly affecting hemostasis, inflammation and immunity are found in the saliva of hematophagous insects. A schematic summary including some of the most common activities found in the saliva of BFIs is provided in Figure 1.

Although the main role of BFI salivary secretions is to allow for an effective acquisition of the blood meal, there are a few additional implications. First, vector-borne pathogens are injected into vertebrate hosts, and exposed to their immune system, along with vector saliva. In virtue of its immunomodulatory properties, BFI saliva can modify the local milieu at the biting site and, as 'side effect', may facilitate the establishment of an infection and affect transmission of vector-borne pathogens [7••,8,9••,10]. Moreover, vertebrate hosts develop an anti-saliva antibody response that can be utilized to assess exposure to vector bites, a tool that may be useful for epidemiological studies, to evaluate control interventions and eventually estimate transmission risk [8,9••,10,11]. For these reasons salivary proteins of blood feeding arthropods (BFAs) combine well basic research interests to translational aspects and may be exploited not only for the development of novel drugs (e.g. antithrombotics), but also as vaccine targets to prevent transmission of vector-borne diseases or as biomarkers of exposure to vectors [7••,8,9••,12,13•].

In this review we will focus on a few recent advances on the understanding of evolution and divergence of salivary genes in BFIs, on the identification of the first insect salivary complement inhibitors and on kratagonists, a recently recognized heterogeneous class of antagonists with a common mechanism of action, that is binding with

Please cite this article in press as: Arcà B, Ribeiro JMC: Saliva of hematophagous insects: a multifaceted toolkit, Curr Opin Insect Sci (2018), https://doi.org/10.1016/j.cois.2018.07.012

Figure 1



Activities most commonly found in the saliva of blood feeding insects. A diagram of a salivating mosquito is shown. Note that the activities indicated are not limited to those found in mosquito saliva and that the list is far from being exhaustive. The main involvement of the specific activities is indicated, however consider that there is extensive cross-talk, especially between hemostasis and inflammation. Anticoagulants, antiplatelets and vasodilators are among the best known and include a large variety of different molecules: enzymes (e.g. apyrases, peroxidases, serine proteases), protease inhibitors, peptides, kratagonists, etc. Contact pathway inhibitors: act on both coagulation and inflammation. Kratagonists: bind small (biogenic amines, eicosanoids) and larger (collagen, heparin, polyphosphate) agonists of hemostasis and inflammation. miRNAs: recently reported in mosquito saliva, their role it is presently not known. Complement inhibitors: protect the insect from host complement, may inhibit inflammation at the bite site. Other immunomodulators: act on different components of the host immune system, only relatively few activities characterized in detail. Other enzymes: besides enzymes acting on hemostasis (e.g. apyrases, fibrinogenolytic) or as antibacterials (lysozyme) there are several additional enzymatic activities in saliva, for example glycosidases (sugar digestion), proteases, hyaluronidases and endonucleases (may help the diffusion of other salivary components at the bite site by hydrolizing extracellular matrix components or DNA released from damaged cells). Mucins: possibly involved in lubrication of mouthparts. Lysozyme, pattern recognition molecules and antimicrobial peptides: involved in antibacterial activity and insect innate immunity. More comprehensive overviews can be found in [5,6,7••].

high affinity mediators of hemostasis and inflammation. Finally, will briefly report on the finding that saliva of BFIs also carries microRNAs, whose possible functions and potential implications in vector–host–pathogen interactions are still to be elucidated.

Complexity of sialomes of blood feeding insects

Transcriptomic, proteomic and genomic studies performed in the last decade greatly contributed to extend our understanding of complexity, function and evolution of salivary secretions of BFIs. Transcriptome studies on 49 hematophagous insect species belonging to 3 orders (Diptera 35 species, Hemiptera 12 species, Siphonaptera 2 species), 11 families and 21 genera are currently available (Table 1). Extracting the number of proteins making up the sialomes (from the Greek *sialo* = saliva) of different BFI families is not straightforward due to differences in sequencing technology (Sanger versus Illumina) and deepness among these studies. However, as a tentative rough estimation we could say that fleas and most blood feeding Nematocera (mosquitoes, sand flies, black flies) carry in their saliva ~100–200 proteins, Brachycera as tsetse flies and horse flies ~250–300 and kissing bugs more than 300. Differences in the feeding mode (capillary versus pool feeding) and duration (up to 20–30 min in kissing bugs) may have to do with the variation in number of putative salivary proteins in these insect families. A theme emerging from this huge amount of data is the impressive diversity of salivary proteins. In fact, along with proteins and protein families that are widely spread among BFIs there are several examples of family-specific, genus-specific and even species-specific proteins [14•,15]. The independent evolution of hematophagy in different lineages, the task of dealing with a range of different hosts and with their redundant physiological responses to tissue injury, the fast evolutionary rate of salivary genes involved in blood feeding certainly played a major role in shaping this remarkable diversity [6]. Noteworthy, a rather large number of putative salivary polypeptides identified to date (~30–40%) do not show similarity to any known protein, indicating that several additional activities are still to be discovered and that the complexity of sialomes of BFIs is even higher than we can presently appreciate.

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