Ecological genomics of sand fly salivary gland genes: An overview

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ABSTRACT: Sand fly saliva contains an array of bioactive molecules that facilitate blood feeding and also function as modulators of the vertebrate immune response. Such a complex of biologically active molecules was shown to be both conserved and divergent among sand fly species. It is likely that expression of sand fly salivary molecules could be modulated by environmental factors, both biotic and abiotic, that ultimately dictate the quality, and possibly quantity, of the secreted saliva. Carbohydrates are an integral part of the sand fly diet, and sugar-sources found in natural habitats are potentially involved in defining the profile of sand fly saliva, and may influence vectorial capacity. Saliva can drive the outcome of Leishmania infection in animal models, and salivary molecules are potential targets for development of vaccines to control Leishmania infection. Thus, identifying what environmental factors effectively modulate sand fly saliva in the field is a critical step towards the development of meaningful protection strategies against leishmaniasis that are based on salivary compounds from sand fly vectors. Journal of Vector Ecology 36 (Supplement 1): S58-S63. 2011.

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INTRODUCTION

Sand fly saliva is a strong modulator of the host immune response and capable of enhancing transmission of Leishmania (Titus and Ribeiro 1988, Belkaid et al. 2000, Kamhawi et al. 2000). The enhancing effects of sand fly saliva are correlated with its ability to inhibit macrophage functions, such as antigen presentation and nitric oxide (NO) synthesis by the infected cells (Belkaid et al. 2000, Titus et al. 2006). In contrast, immunity to salivary components can prevent establishment of infection (Kamhawi et al. 2000, Valenzuela et al. 2001, Gomes et al. 2008, Collin et al. 2009). Due to the crucial role played by sand fly saliva during transmission of Leishmania to vertebrate hosts, and to the possibility of using saliva or salivary components to protect against these parasites, understanding the role of individual salivary proteins is of great importance for the development of salivary components-based vaccines (Oliveira et al. 2006).

The salivary transcriptomes of several sand fly species have been identified, providing an insight on the types of molecules inoculated into host skin by these vectors. Further, at least for some salivary secreted proteins, their roles in protecting animals against challenge with Leishmania have been ascertained (Kamhawi et al. 2000, Valenzuela et al. 2001, Gomes et al. 2008, Collin et al. 2009). In spite of the efforts to identify salivary proteins in sand fly saliva, few studies have focused on the variability of saliva and salivary components in sand flies (Lanzaro et al. 1999, Eliaieim et al. 2005, Anderson et al. 2006, Kato et al. 2006), and none of which has focused on potential effects of environmental factors.

In this review, we discuss some of the underlying environmental factors that may be associated with the gene expression plasticity in sand flies and outline how such plasticity can influence aspects of sand fly physiology. In addition, the potential involvement of seasonal expression profiles of salivary gland genes on pathogen transmission and epidemiology of leishmaniasis also is exploited.

Sand fly saliva

Since the seminal study by Titus and Ribeiro (Titus and Ribeiro 1988) demonstrating the effect of sand fly saliva exacerbating lesion development in mice injected with Le. major, many studies have focused on the role of saliva and salivary molecules in pathogen transmission. For sand fly-transmitted leishmaniasis, it became evident that the strong immunomodulatory effects of the vector saliva contribute to the establishment of the parasite and the onset of disease. However, pre-exposure to saliva and salivary gland homogenates can lead to protection against challenge with Leishmania, supporting the assumption that saliva may be used in vaccinations strategies.

For P. papatasi, the principal vector of Le. major in North Africa and in the Middle East, transcriptome analyses of salivary glands of female sand flies revealed a total of roughly 35 predicted secreted salivary proteins. One such protein, PpSP15, was shown to confer protection to mice against challenge with Le. major (Valenzuela et al. 2001). This protection was observed following natural (infected sand fly bites) or artificial (needle injection) challenge with Le. major (Valenzuela et al. 2001). However, the protection conferred by PpSP15 has to date only been confirmed for mice, as this effect was not observed for this molecule when Rhesus monkeys were used (Valenzuela, personal communication.). Thus, different salivary molecules are associated with protection against Leishmania in different vertebrate hosts (Gomes et al. 2008, Collin et al. 2009).

Interestingly, SP15 constitute a family of proteins present in other species of sand flies, including P. duboscqi, P. ariasi, and P. arabicus (Anderson et al. 2006, Kato et al. 2006, Oliveira et al. 2006, Hostomska et al. 2009), and a SP15-like
molecule also was found in *Lu. longipalpis* (Charlab et al. 1999, Valenzuela et al. 2004).

For humans, no single *P. papatasi* (or from other sand fly species, for that matter) salivary molecule has been identified that might lead to protection. However, in line with the argument that salivary components may be targets for vaccine development against *Leishmania* and due to the fact that the majority of studies undertaken relied on long term laboratory-reared sand fly colonies, El-naiem et al. (2005) investigated the variability of SP15 in natural and colonized populations of *P. papatasi*. The results obtained suggested some degree of variability for PpSP15 in which wild-caught *P. papatasi* displays higher genetic levels of variation than colonized flies (El-naiem et al. 2005).

Over the last twenty years, many investigators have relied on colonized sand flies to investigate the effect of saliva on transmission of *Leishmania* to animal models. However, recent data suggest that some of the effects detected might have been due to changes in saliva following the colonization process. In one set of studies the effects of salivary gland homogenate (SGH) from wild-caught *Lu. longipalpis* were shown to vary greatly from those produced by laboratory-reared flies (Laurenti et al. 2009a, Laurenti et al. 2009b). Mice inoculated in the ear or foot pad with 10⁶ *Le. amazonensis* with SGH from wild-caught flies displayed smaller lesions, and macrophages infected by parasites were fewer than those isolated from mice inoculated with SGH from laboratory-reared flies (Laurenti et al. 2009b). In addition, the immunomodulatory effects of these two SGH also were different: wild-caught SGH led to lower production of IL-4 and IL-10 but higher IL-12 levels compared with laboratory-reared SGH (Laurenti et al. 2009a). In separate studies involving *P. papatasi*-transmitted *Le. major*, pre-immunization of mice with SGH obtained from long-term laboratory colonies (F29) induced protection against *Le. major* co-inoculated with the same type of SGH, yet it did not confer protection against inoculation with SGH of wild-caught specimens (Ben Hadj Ahmed et al. 2009). Moreover, pre-immunization of mice with SGH of wild-caught female *P. papatasi* did not confer protection against *Le. major* co-inoculated with the same type of SGH (Ahmed et al. 2010).

In addition to the effects of SGH described above, protein sequence polymorphisms as well as differences in the amounts of salivary proteins also were tentatively associated with different disease outcomes (Warburg et al. 1994, Lanzaro et al. 1999, Yin et al. 2000, Morris et al. 2001, Milleron et al. 2004). Specifically, differential expression of maxadilan transcripts between cryptic species of the New World sand fly *Lu. longipalpis* is believed to be involved in visceralization of *Le. infantum chagasi* transmitted by this sand fly as it correlates with the different clinical manifestations of leishmaniasis in Central and South America (Warburg et al. 1994, Lanzaro et al. 1999, Yin et al. 2000). While in Brazil and Colombia *Lu. longipalpis*-transmitted *Le. i. chagasi* causes visceral leishmaniasis, in Costa Rica, Nicaragua, and Honduras it leads to atypical cutaneous lesions (Zeledon et al. 1984, Zeledon et al. 1989, Warburg et al. 1994, Carrasco et al. 1998, Belli et al. 1999). Experimentally, lesions obtained by needle injection of *Leishmania* in mice also correlated with amounts of co-injected maxadilan (Morris et al. 2001).

Together, these data underscore the significant role played by sand fly genetic divergence in modulating the quantity of sand fly saliva inoculated into host skin, leading to different disease outcomes.

**Genetic structure of *P. papatasi***

In spite of data suggesting that *P. papatasi* is not a species complex (Hamarsheh et al. 2007, Depaquit et al. 2008), previous studies demonstrated physiological, behavioral, and genetic differences between *P. papatasi* populations from different geographic localities (Wu and Tesh 1990b, Hanafi et al. 1998). Susceptibility to infection with *Le. major* also was shown to differ between colonized flies originally from Israel, India, and Egypt, whereby the Israeli strain (PPIS) displayed the highest rate of infection (Wu and Tesh 1990a). Distinct infection levels also were detected in the laboratory with *P. papatasi* from different locations in Egypt (Hanafi et al. 1998).

Recent analysis of polymorphisms in the cytochrome b gene (*cyt b*) from colonized and natural populations of *P. papatasi* revealed moderate genetic differentiation between populations from Egypt and Middle East (Hamarsheh et al. 2007). These results contrasted those obtained for the internal transcribed spacer 2 (*ITS2*) and NAD dehydrogenase subunit 4 (*ND4*) genes that pointed to a lack of genetic structuring across the *P. papatasi* geographical range (Depaquit et al. 2008). Thus, despite the levels of genetic similarities among *P. papatasi* populations, specific trait differences (physiological and/or behavioral) exist, which might have been shaped by environmental pressures.

The vectorial capacity of *P. papatasi* was previously associated with hunger tolerance (Schlein and Jacobson 2001, Schlein and Jacobson 2002). Thus, selective pressure from ecological factors can have a significant impact on the evolution of *P. papatasi* genes associated with vectorial capacity, such as salivary gland- and midgut-expressed genes. Much is known about the ecology of *P. papatasi* in the Middle East and North Africa (Schlein et al. 1982a, Schlein et al. 1982b, Yuval and Schlein 1986, Schlein and Yuval 1987, Yuval et al. 1988, Yuval 1991, Schlein and Jacobson 1994, Janini et al. 1995, Schlein and Jacobson 1999, Schlein and Jacobson 2000, Schlein and Jacobson 2001, Schlein and Jacobson 2002, Chelbi et al. 2007, Zhioua et al. 2007, Chelbi et al. 2009). Nevertheless, how the environment influences gene expression in this sand fly is vastly unexplored.

**Environmental effects on *P. papatasi* gene expression**

Ecological genomics seeks to uncover the genetic mechanisms that respond to environmental changes (Ungerer et al. 2008). The effects of the environment on the gene expression are frequently referred to as genotype-by-environment interaction, and the responses displayed by organisms to such environmental change are named phenotypic plasticity (Gibson 2008). Differential gene
expression or gene expression plasticity can be triggered by biotic or abiotic factors (Hodgins-Davis and Townsend 2009), and gene expression can be more strongly correlated with the environment than with genetic divergence (Whitehead and Crawford 2006). Thus, selection by the environment can fine-tune gene expression for higher fitness (Hodgins-Davis and Townsend 2009).

Ecological studies on *P. papatasi* have addressed important questions about which environmental factors can influence *P. papatasi* vector competence (Schlein and Jacobson 1994, Schlein and Jacobson 2001, Schlein and Jacobson 2002). Sugar sources and water availability in natural habitats (which modulates photosynthesis and in turn plant sugar levels) can modulate expression of vector competence-associated genes. So far, only the influences of the latter factor on sand fly gene expression have been exploited.

*P. papatasi* caught in arid and irrigated habitats, and at different periods of the *P. papatasi* season, exhibited different rates of activity for glycosidases and chitinases (Jacobson et al. 2007), two enzymes possibly involved in vector competence. Our own studies suggest similar seasonal variations for salivary gland transcripts in *P. papatasi* collected in the Middle East. For sand flies collected in a natural habitat (a site without any irrigation system), a gradual increase in the abundance of these mRNAs was detected, with the highest levels assessed late in the sand fly season – September (Coutinho-Abreu et al. unpublished data). This upregulation in salivary gland gene expression, as well as in glycosidase and chitinase enzymatic activities (Jacobson et al. 2007), coincided with the fact that this non-irrigated area becomes even dryer late in the season and with scarce sugar sources for sugar feeding insects, such as sand flies (Schlein and Jacobson 2000).

The finding that sap from plants in dry and irrigated habitats varies in sugar concentration (Schlein and Jacobson 2002) suggests that availability of sugar sources is one of the factors responsible for the differential expression of salivary gland genes exhibited throughout the season. On the other hand, other biotic (and abiotic) factors might also influence the expression profile of *P. papatasi* salivary gland genes, such as senescence and pregnancy. Phenology studies demonstrated that *P. papatasi* populations from different habitats can exhibit differences in the percentages of gravid or engorged females, as observed in the Jordan Valley (Yuval 1991, Janini et al. 1995). Additionally, *P. papatasi* parous rate also may vary (Yuval 1991, Hanafi et al. 2007), suggesting that sand flies are older late in the season. However, our results from a study on the influences of aging and gonotrophic stage on the expression of salivary gland genes in colonized *P. papatasi* do not broadly support this hypothesis. In our analyses, *PpSP44* was the only transcript amongst the ten most abundant salivary transcripts investigated that was influenced by such factors (Coutinho-Abreu et al. 2010).

### P. papatasi gene expression plasticity and Leishmania transmission

For some endemic sites of transmission of zoonotic cutaneous leishmaniasis (ZCL), such as Tunisia, the entomological risk index (ERI), associating the infection rate of *P. papatasi* with *Le. major* and the abundance of the vector, is related for the most part with the geographical distribution of the disease (Chelbi et al. 2007). However, such correlation between ERI and ZCL is not necessarily found at all ZCL endemic sites (Chelbi et al. 2009). Several reasons may explain this epidemiological behavior for the disease, including greater rate of infectious bites shown to be highest in the fall (Chelbi et al. 2009). Nevertheless, another potential explanation for such large incidence of parasites, both in terms of high number of infected reservoir as well as human cases, may be associated with changes in the salivary gland profile of sand flies. Higher dose of saliva inoculated into the host skin may modulate *Leishmania* development, as observed in animal model in laboratory (Morris et al. 2001).

### CONCLUDING REMARKS

In our view, sand flies are an important model for studies focusing on the influence of the environment on vectorial capacity of disease vectors. *P. papatasi* simple ecotone in Middle East deserts, where inter-specific competition and trophic level interactions are limited, makes this vector a good choice for studies focused on ecological genomics, sand fly behavior, and impact on epidemiology of ZCL. Pioneering work revealing the influences of biotic and abiotic components on *P. papatasi* vectorial capacity provides the basis for the ecological genomics hypotheses addressing the influences of ecological factors on genes associated with sand fly vectorial capacity. In regards to *P. papatasi* salivary gland genes, expression levels are likely fine-tuned based upon the levels of water available in the environment. Although we still do not know the molecular basis for the *P. papatasi* gene expression differences (and different levels of enzymatic activity) in seasonally changing environments, plant carbohydrates change according to water availability. Accordingly, sand flies would need to adjust their saliva in order to obtain more nutrients from such plants and improve their fitness). Nevertheless, the role of sand fly saliva on sugar feeding remains vastly unexplored (Calvo et al. 2006).

Overall, the assessment of *P. papatasi* salivary gland gene expression in a changing environment has significant impact for the sand fly saliva-based vaccine studies. In addition, it opens new avenues for future studies of environment influences on the expression of other genes related with vectorial capacity, such as genes affecting host and plant-seeking behavior as well as vector-parasite interactions.
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REFERENCES CITED


