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Rhodnius prolixus supergene families of enzymes potentially associated with insecticide resistance

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ABSTRACT

Chagas disease or American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi*. Once known as an endemic health problem of poor rural populations in Latin American countries, it has now spread worldwide. The parasite is transmitted by triatomine bugs, of which *Rhodnius prolixus* (Hemiptera, Reduviidae, Triatominae) is one of the vectors and a model organism. This species occurs mainly in Central and South American countries where the disease is endemic. Disease prevention focuses on vector control programs that, in general, rely intensely on insecticide use. However, the massive use of chemical insecticides can lead to resistance. One of the major mechanisms is known as metabolic resistance that is associated with an increase in the expression or activity of detoxification genes. Three of the enzyme families that are involved in this process – carboxylesterases (CCE), glutathione s-transferases (GST) and cytochrome P450s (CYP) – are analyzed in the *R. prolixus* genome. A similar set of detoxification genes to those of the Hemipteran *Acyrthosiphon pisum* but smaller than in most dipteran species was found in *R. prolixus* genome. All major CCE classes (43 genes found) are present but the pheromone/hormone processing class had fewer genes than usual. One main expansion was detected on the detoxification/dietary class. The phosphotriesterase family, recently associated with insecticide resistance, was also represented with one gene. One microsomal GST gene was found and the cytosolic GST gene count (14 genes) is extremely low when compared to the other hemipteran species with sequenced genomes. However, this is similar to *Apis mellifera*, a species known for its deficit in detoxification genes. In *R. prolixus* 88 CYP genes were found, with representatives in the four clans (CYP2, CYP3, CYP4 and mitochondrial) usually found in insects. *R. prolixus* seems to have smaller species-specific expansions of CYP genes than mosquitoes and beetles, among others. The number of *R. prolixus* CYP genes is similar to the hemipteran *Ac. pisum*, although with a bigger expansion in CYP3 and CYP4 clans, along with several gene fragments, mostly in CYP4 clan. Eleven founding members of new families were detected, consisting of ten genes in the CYP3 clan and 1 gene in the CYP4 clan. Members of these clans were proposed to have important detoxification roles in insects. The identification of CCE, GST and CYP genes is of utmost importance for directing detoxification studies on triatomines that can help insecticide management strategies in control programs.

1. Introduction

Chagas disease, or American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi*. Recent estimates indicate that about seven million

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people around the world might be infected (Punta et al., 2014; World Health Organization, 2014). Once known as an endemic health problem of poor rural populations in Latin American countries, where the primary insect vectors live, it has now spread worldwide with as many as 300 thousand infected individuals in the USA alone (Bern and Montgomery, 2009; CDC, 2013; Coura and Viñas, 2010). Because of growing population movements, Chagas disease is now a worldwide concern that can have severe consequences in the long term. The economic costs associated with disability and mortality of this disease are enormous and will continue to grow because of the high proportion of infected people that will develop the chronic form (Rassi et al., 2010; World Health Organization, 2008).

The parasite can be transmitted through blood-sucking triatomine bugs, contaminated food or transfusion of infected blood (World Health Organization, 2014). The disease is endemic in most Latin American countries where it is mainly vector-borne transmitted by contact with feces of some species of triatomine bugs (kissing bugs), of which *Rhodnius prolixus* (Hemiptera, Reduviidae, Triatominae) is one of the vectors and a model organism. *R. prolixus* occurs mainly in Central and South American countries where Chagas disease is endemic and a very serious health problem.

Classified as a tropical neglected disease, this illness does not receive attention from pharmaceutical and biotechnology industries and, consequently, there is little expectation for new drugs, diagnostic kits or even a vaccine. In this scenario, disease prevention focuses on vector control programs that, in general, rely intensely on chemical control (Moncayo and Silveira, 2009; World Health Organization, 2014). Some progress in containing disease transmission has been attained (Dias et al., 2002; Moncayo and Silveira, 2009) and *R. prolixus* has been recently considered eradicated from Central America (Hashimoto and Schofield, 2012). However, it is possible that other triatomine bugs may take its place. One of the reasons why species of Reduviidae have been important vectors is that most of them are very effective in adopting a domestic lifestyle, with *Rhodnius* being the most efficient one (Schofield et al., 1999). However, a sylvatic life cycle of *T. cruzi* has involved more than 150 species of triatomines and another 100 wild mammals for millions of years (Coura and Dias, 2009; Coura and Viñas, 2010).

R. prolixus is also an important model species and is responsible for many insights into the hematophagous Reduviidae life cycle, basic biology and development. The *R. prolixus* genome has recently been sequenced and is available for comparative studies with other insect species. These studies might help us better understand triatomine life cycle and evolution and identify new targets for insecticide development thus aiding the control of pathogen transmission.

Although it is expected that new vector control strategies should be developed for all insect species (McGraw and O'Neill, 2013), chemical control still plays an important part on integrated pest management programs. The massive use of chemical products can lead to insecticide resistance, as has happened with many insect vectors (Bass and Field, 2011; Li et al., 2007). In *Triatoma infestans*, the major Chagas disease vector in southern South America, for example, resistance to deltamethrin and other insecticides has been reported in different areas of the Gran Chaco region of Argentina and Bolivia (Germano et al., 2010; Picollo et al., 2005). Although only one study has documented insecticide resistance in *R. prolixus* (Vassena et al., 2000), parallel evolution seems to be especially common in this phenotypic trait (ffrench-Constant, 2013). Therefore, monitoring of insecticide resistance and the mechanisms involved is of critical importance.

Two major types of insecticide resistance mechanisms have been observed in natural populations: point mutations in insecticide targeted genes and over-expression or mutations in genes encoding detoxification enzymes. The latter, also known as metabolic resistance, implies a greater capacity to detoxify insecticides due to an increase in the expression or in the activity of genes related to detoxification metabolism. Four enzyme families – esterases, glutathione s-transferases (GST), cytochrome P450s (CYP) and, more recently, ABC transporters have been implicated in insecticide resistance and are therefore highly relevant to public health issues (Bass and Field, 2011; Dermauw and Van Leeuwen, 2014; Li et al., 2007).

In this study we investigate the gene content of three major detoxification enzyme families (esterases, GSTs and CYPs) in the *R. prolixus* genome. We compare both the identity and the number of genes found with other insect species whose genome sequences are available. Phylogenetic analysis was used to infer gene classification and evolution. *R. prolixus* exhibits a diverse set of genes encoding detoxification enzymes. In each family, gene numbers in some classes (or 'clans', according to the usual nomenclature adopted for CYPs) are similar to those of other hemipteran species while others resemble the honeybee *Apis mellifera* that has a genome depleted of detoxification genes. Comparative genomics of enzyme families conserved across organisms helps not only in gene annotation but also in unraveling their evolutionary structure and function. The identification of all these genes is of utmost importance as basic knowledge and also to direct detoxification studies on triatomines that can help insecticide management strategies in control programs.

2. Material and methods

2.1. Identification of R. prolixus detoxification enzymes

Sequences encoding GSTs and esterases were identified from the predicted protein set of *R. prolixus* (version Rhodnius-prolixus-CDC_PEPTIDES_RproC1.2), *Drosophila melanogaster* (version dmel-all-translation-r5), *Acyrthosiphum pisum* (version aphidbase_v2_pep), *Ap. mellifera* (version Apismellifera4) and *Anopheles gambiae* (version Anopheles-gambiae-PEST_PEPTIDES_AgamP3) using the FAT program (Seabra-Junior et al., 2011) with their respective protein family domains. The carboxylesterase (CCE) PF00135, GST PF00043 and PF02798 and phosphotriesterase (PTE) PF02126 domains were retrieved from the Pfam database (Punta et al., 2014). All proteins with significant E-value (<0.0001) were retrieved and a first manual analysis of *R. prolixus* predicted peptides was performed with BLASTp searches (Altschul et al., 1990) against the manually curated Uniprot/SwissProt protein database (The UniProt Consortium, 2014) in FAT. The BLASTp tool on VectorBase and NCBI websites was also used to search for homologues of *R. prolixus* genes in other well-annotated species. For *Ap. mellifera*, *An. gambiae*, *D. melanogaster* and *Ac. pisum* the predicted proteins found in the domain search were not inspected further. These species were used to help identify the different *R. prolixus* genes since most of their genes are already well annotated. In some cases, for these four species, different protein transcripts for the same gene were used and therefore the number of genes in the tree might not match the gene table where data was retrieved from other published works. Predicted peptides were downloaded from VectorBase (http://www.vectorbase.org/), FlyBase (http://flybase.org/), AphidBase (http://www.aphidbase.com/) and Hymenoptera Genome Database (http://hymenopteragenome.org/). For the GSTs, genes with classification obtained from other three hemipterans, taken from the work of Zhou et al. (2013), were also used. To search the *R. prolixus* genome for microsomal and kappa GSTs, already annotated sequences from human, *Ac. pisum* and *Caenohrabiditis*

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