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REVIEW

Genetics and evolution of triatomines: from phylogeny to vector control

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Triatomines are hemipteran bugs acting as vectors of the protozoan parasite *Trypanosoma cruzi*. This parasite causes Chagas disease, one of the major parasitic diseases in the Americas. Studies of triatomine genetics and evolution have been particularly useful in the design of rational vector control strategies, and are reviewed here. The phylogeography of several triatomine species is now slowly emerging, and the struggle to reconcile the phenotypic, phylogenetic, ecological and epidemiological species concepts makes for a very dynamic field. Population genetic studies using different markers indicate a wide range of population structures, depending on the triatomine species, ranging from highly fragmented to mobile, interbreeding populations. Triatomines transmit *T. cruzi* in the context of complex interactions between the insect vectors, their bacterial symbionts and the parasites; however, an integrated view of the significance of these interactions in triatomine biology, evolution and in disease transmission is still lacking. The development of novel genetic markers, together with the ongoing sequencing of the *Rhodnius prolixus* genome and more integrative studies, will provide key tools to expanding our understanding of these important insect vectors and allow the design of improved vector control strategies.
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TRIATOMINES AND CHAGAS DISEASE

Chagas disease is one of the most neglected tropical diseases, named after the Brazilian physician Carlos Chagas who first described it 100 years ago. It is caused by the protozoan kinetoplastid parasite *Trypanosoma cruzi*, which is transmitted to mammalian hosts primarily by blood-feeding insect vectors of the Reduviidae family. *T. cruzi* infection affects 9–11 million people, mostly in Latin America, but international migration means it is now a global disease reaching vector-free areas (WHO, 2007; Gurtler *et al.*, 2008; Jackson *et al.*, 2009).

Because of limited therapeutic options, most control efforts have focused on the elimination of triatomine vectors from houses, usually by application of residual insecticides and/or housing improvement (Dias *et al.*, 2002). Research devoted to understanding of triatomine biology, genetics and evolution has supported rational vector control strategies; the latter two are reviewed here.

Triatomines are hemipteran (true) bugs, which go through five nymphal stages before reaching the adult stage. At least one blood meal is required to molt into the next stage. The development from egg to adult lasts 3–6 months depending on the species and feeding frequency, and insects can live from 6 months to 2 years (Beard, 2005). Triatomines become infected when they feed on a mammalian host infected with *T. cruzi*. The parasites then multiply in the insect's guts and can be transmitted to a new host during a subsequent blood meal when accompanied by defecation of the vector as infective parasites are passed in the feces. The parasite can then enter the mammalian host through microlesions of the skin or through mucous membranes (Beard, 2005).

Some triatomine species have been able to adapt to human housing, becoming domiciliated, and are thus very effective vectors of Chagas disease owing to the extensive contacts with humans. Others have remained more sylvatic (remaining in wild areas), and only occasionally invade houses and may then feed on humans (Beard, 2005).

SYSTEMATICS AND PHYLOGENY

Most triatomine species (subfamily Triatominae) and 14 of the 15 known genera occur in the New World (Figure 1); one genus is unique to Asia and the genus *Triatoma* is found both in Asia and America. The evolution and diversification of the Triatominae is thought to have coincided with that of mammals and birds during the Jurassic period (Schofield, 2000, Tartarotti *et al.* 2006). They belong to the Reduviidae (Heteroptera), a much older family of predatory bugs (already present in the Permian–Triassic period) with a worldwide distribution that feeds on other insects rather than sucking blood (Schofield, 2000). It is believed that the evolution of the Cimicidae blood-sucking bugs in Africa preceded that of Triatominae in America and thus prevented their expansion on the African continent by occupying their potential ecological niche (Tartarotti *et al.* 2006).

The task of classifying the currently 140 recognized triatomine species and understanding their evolutionary relationships is not

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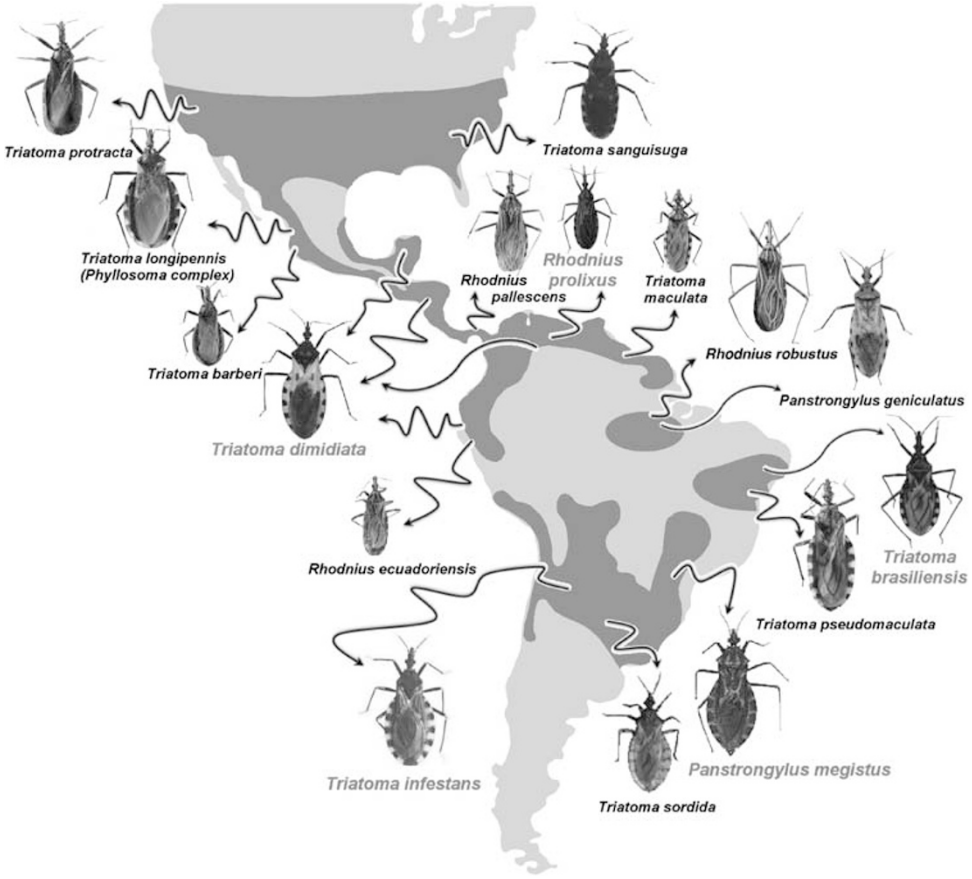

Figure 1 Distribution of triatomine species of major epidemiological relevance. Only about 20 triatomine species are responsible for *T. cruzi* transmission to humans, because of their ability to infest and, for some species, to colonize human habitat. The red areas indicate the approximate species geographic distribution. Species highlighted in red are considered the most important vectors of the parasite.

simple (Schofield and Galvão, 2009). It is important to stress that, because triatomine research is driven by their medical importance, the focus is often on 'epidemiological types' because they are more relevant to Chagas disease epidemiology and vector control than classical taxonomical distinctions. Thus, only about 20 species from the genera *Triatoma, Rhodnius* and *Panstrongylus* are particularly relevant for *T. cruzi* transmission to humans, and most studies focus on these species (Figure 1). Epidemiologically, *Triatoma infestans*, *Triatoma dimidiata*, *Triatoma brasiliensis*, *Rhodnius prolixus* and *Panstrongylus megistus* are considered the most important primary vectors. The reconciliation of the phenotypic, phylogenetic and ecological/epidemiological species concepts, and application of distinct methodologies, makes for a very dynamic field of research in medical entomology.

Triatomine species identification: interbreeding, cytotypes and species complexes

Because of differences in vectorial capacity among triatomine species, their correct taxonomic identification is a first key step for vector surveillance and control programs. The current classification of the Triatominae into five tribes and 15 genera is largely based on morphological characters, including chromatic ones (Lent and Wygodzinsky, 1979). The most commonly used characters are head and wing shape (Dujardin *et al*., 1999b; dos Santos *et al*., 2003; Dumonteil *et al*., 2007), but a number of other characters have been used such as the structure of the male genitalia (Obara *et al*., 2007), the egg (dos Santos *et al*., 2009), antenna (Silva *et al*., 2002; Catala *et al*., 2005) and rostrum (Silva *et al*., 2003). Within the triatomines, for example, the monophylly of the Rhodniini tribe has been supported by apical antenna insertion, body forms, post-ocular callosities, male genital characteristics, egg surface architecture and salivary gland protein composition (Tartarotti *et al*., 2006).

Morphological characters can distinguish the vast majority of triatomine species (Carbajal de la Fuente *et al*., 2011). Recently diverged species can sometimes be differentiated (Gurgel-Goncalves *et al*., 2010) or be remarkably isomorphic, making the detection and description of cryptic taxa difficult (Abad-Franch and Monteiro, 2005; Nouvellet *et al*., 2011). A good example of the underlying taxonomic structure, which could not be resolved by morphological studies alone, is the *Triatoma sordida* species complex whose Argentinean, Brazilian and Bolivian member species could only be described thanks to additional tools such as cytogenetics and molecular markers (Dujardin *et al*., 1999b). Other triatomine species show such high levels of phenotypic plasticity that they were erroneously split into several species. For example, chromatic variants of *T. infestans* (one of the most important vectors found in Peru, Bolivia, Brazil, Chile, Paraguay, Uruguay and Argentina) were considered a different species (*Triatoma melanosoma* in Argentina) or variants (a 'dark morph' in Bolivia). However, these 'species' or variants produced fertile offsprings with *T. infestans* and no difference was found using cytogenetics and

molecular markers (Noireau *et al*., 1997; Dujardin *et al*., 1999a; Monteiro *et al*., 1999; Bargues *et al*., 2006). Similarly, revisions are underway in the *Phyllosoma* complex, which includes important vectors species in Mexico, all of which are interfertile, so also suspected of incorrect species-level designations (Martinez-Ibarra *et al*., 2009; Martinez-Hernandez *et al*., 2010).

There are countless examples in which experimental crosses have been used to complement morphological studies when the latter lead to inconclusive results (Perez *et al*., 1992), and these still have an important role in clarifying the specific and sub-specific status in triatomine species complexes. For example, crosses were instrumental in establishing the specific status of *Triatoma pseudomaculata*, an important sylvatic vector in Brazil (Corrêa and Espínola, 1964). They also helped in distinguishing *Rhodnius prolixus* and *Rhodnius robustus*, the main vectors of Chagas disease in Venezuela, Colombia and some other regions of central America (Galíndez-Giron *et al*., 1994). As mentioned previously, crosses were important in clarifying taxonomy in the *Infestans* complex (Abalos, 1948; Franca, 1985; Monteiro *et al*., 1999), and more recently, the *Phyllosoma* complex (Martinez-Ibarra *et al*., 2009; Martinez-Hernandez *et al*., 2010).

As in the Diptera, cytogenetics has been an essential tool in Triatominae systematics since the early 1900s (Payne, 1909) and is still very much in use in combination with molecular markers (Bargues *et al*., 2006; dos Santos *et al*., 2007). An interesting application involves cytogenetics used in combination with crosses to study karyotypic evolution or 're-patterning' (Perez *et al*., 2005).

Triatomines show high chromosome homogeneity; nearly all have 20 autosomes and most South American species show two sex chromosomes (XY). Secondary fragmentation is thought to have led to the few instances of 2 or 3 X-chromosomes (X_1X_2Y and $X_1X_2X_3Y$) (Perez *et al*., 1992) found in some Central and North American species (Panzera *et al*., 2010). Triatomine chromosomes are holocentric (that is, centromeric activity is spread over the entire chromosomal arm) and unlike the large polytenic metacentric chromosomes of the Diptera, which show clear euchromatic banding patterns, they yield very little information in terms of chromosomal polymorphism. Cytogeneticists have concentrated instead on comparing different stages of meiosis between putative species using c-banding staining, which marks constitutive heterochromatin and allows the identification of distinct heterochomatic blocks along chromosomes (Perez *et al*., 1992).

Cytogenetic comparisons have helped clarify the *T. sordida* complex of 'isomorphic' species. They highlighted chromosomal differences between the smaller and darker Argentinean populations of *T. sordida* (previously known as *Triatoma garciabesi*) and Brazilian populations, as well as between *T. sordida* and other closely related species of the complex (Panzera *et al*., 1997).

Interestingly, chromosomes are often marked by intraspecific polymorphism in heterochromatic content (Panzera *et al*., 1992; Panzera *et al*., 2004). For example, the Andean populations of *T. infestans* present a 50% higher constitutive heterochromatin content than the non-Andean populations. This led to the suggestion that recent colonization of new habitats has been accompanied by genome reduction (Panzera *et al*., 2004), but intermediate forms have been described in northern Argentina, which complicates phylogeographic interpretation (Monteiro *et al*., 2010). In recent studies, measurements of haploid genome size have been used to complement c-banding karyotype comparisons (Panzera *et al*., 2004) or to corroborate molecular data (Bargues *et al*., 2006).

Cytogenetics also had a crucial role in the ongoing description of *T. dimidiata*. This species presents morphologically variable populations, but early attempts to delineate geographic boundaries between them failed, and clinal variation was proposed to explain this species' extensive polymorphism (reviewed by Dorn *et al*., 2007). Later on however, a combination of morphology, cytogenetics and molecular data led to the unambiguous detection of a cryptic taxon and the recognition of the *Dimidiata* species complex (Marcilla *et al*., 2001; Panzera *et al*., 2006; Dorn *et al*., 2007, 2009).

In the 1980s, isozyme analysis was first applied to population genetic questions of triatomines. Fixed allelic differences characterizing certain sympatric populations were quickly recognized as proof of their reproductive isolation, hence potential specific status. A good example was the 'chance' discovery of two reproductively isolated sylvatic populations of *T. sordida* in a study of genetic divergence between *T. sordida* and a closely related species, *T. guasayana* (Noireau *et al*., 1998). Another fine example was the realization of the close relationship between the small *Rhodnius ecuadoriensis* and its 'large cousin' *R. prolixus* from Colombia, which morphological studies of the Rhodnius sub-tribe described as unrelated (Chavez *et al*., 1999; Dujardin *et al*., 1999a, b). Isozymes are still used, mostly because of their affordability and have recently helped clarify species complexes such as the *Triatoma rubrovaria* and the *Triatoma maculata* complexes, which include several morphologically similar sylvatic vectors distributed throughout Brazil and Northern South-America (dos Santos *et al*., 2007).

Origin of triatomines: monophyly versus polyphyly

Since the late 1990s, DNA sequence data have been used to test phylogenetic relationships between putative taxa, complementing morphological studies based on morphology. Morphological and molecular phylogenies are often used to validate one another and they can generate remarkably similar trees particularly at higher taxonomic levels (Weirauch, 2008; Weirauch and Munro, 2009). However, at lower taxonomic levels, the extensive plasticity of some species can result in morphological convergence or divergence, thus complicating taxonomic assignations (Lent and Wygodzinsky, 1979; Dujardin *et al*., 1999b). This illustrates the importance of using as many characters as possible for robust taxonomies. A recent cladistic analysis of the assassin bugs (Reduviidae), which includes the Triatominae as well as non-hematophagous families, used as many as 162 morphological characters (Weirauch, 2008). DNA sequences are not immune to convergence (homoplasy) and phylogenetic analyses may depend on the number and types of sequence used, the amount of polymorphic sites they include and a variety of assumption about the rates of mutations (insertions, deletions, transitions, transversions), although these can be estimated independently. Importantly, the possibility of introgression may lead to gene phylogenies that do not reflect the species phylogeny. Future phylogenetic analyses are likely to include whole or nearly whole genomes, and thus provide insights into historical hybridization events.

Perhaps the best illustration of the complexity of phylogenetic interpretations is the long-lasting question of monophyly versus polyphyly of the Triatominae. Morphological studies have been unable to establish if, within the Triatominae, the Rhodniini and the Triatomini tribes evolved from one or more than one bloodsucking ancestor, and whether the *Rhodnius* and *Triatoma* genera are paraphyletic with respect to other genera of the Triatominae. The initial sequence-based studies could also not clearly reject one or the other hypothesis because of lack of statistical power (Hypsa *et al*., 2002; Schofield and Galvão, 2009). Nonetheless, they illustrated the potential pitfalls of phylogenies based on sequence data and led to a progressive increase in the number of taxa, sequence data and analytical approaches involved.

The latest phylogenetic studies of the Triatominae and other Reduviidae combine several nuclear and mitochondrial DNA (mtDNA) sequences, comprise large numbers of taxa and explore extensive parameter spaces by using parsimony, maximum likelihood and Bayesian analyses (Weirauch and Munro, 2009; Patterson and Gaunt, 2010). The resulting trees recover the Triatominae as a monophyletic taxon with good statistical support, particularly from Bayesian and maximum likelihood inferences (Weirauch and Munro, 2009; Patterson and Gaunt 2010).

Additionally, sequence data have been used to clarify taxonomy at different levels. The phylogeny and taxonomy of the Rhodniini tribe, for example, was derived early on from mitochondrial and nuclear sequences (Monteiro *et al*., 2000). Ribosomal DNA in combination with haploid genome size comparisons was used to clarify the *Infestans* complex (Bargues *et al*., 2006). Recent studies based on ribosomal and mitochondrial genes have helped described several putative species and sub-species within the *Dimidiata* complex (Marcilla *et al*., 2001; Ramirez *et al*., 2005; Bargues *et al*., 2006; Dorn *et al*., 2007, 2009; Tamay-Segovia *et al*., 2008).

Sequence data have several other important advantages over other markers. First, they document sequence polymorphisms that can be readily used to develop simple high-throughput PCR diagnostics for discriminating putative taxa, thus greatly facilitating the work with recently diverged isomorphic species group (Pavan and Monteiro, 2007; Herrera-Aguilar *et al*., 2009). Cryptic species are not common in the Triatominae, however they have been detected in *T. dimidiata* (Marcilla *et al*., 2001; Panzera *et al*., 2006), *T. sordida* (Noireau *et al*., 1998; Panzera *et al*., 2006) and *R. robustus* (Monteiro *et al*., 2003). Natural hybrids also occur, for example, between *T. infestans* and *Triatoma platensis*, and these appear to remain separated by occupying distinct ecological niches (Abalos, 1948). In the case of *T. dimidiata* and the cryptic species, decreased hybrid fitness (Herrera-Aguilar *et al*., 2009) may be what is maintaining the >5-million-year separation, as determined using an *ITS-2* molecular clock (Bargues *et al*., 2008). *R. robustus* was a concern as a possibly epidemiologically important sylvatic species nearly identical in appearance to *R. prolixus* (Monteiro *et al*., 2003).

Second, when phylogenies are combined with the known age of geological events to build a so-called 'molecular clock', one can match the age of a putative tree branching event with geological events to generate phylogeographic scenarios. These elegant studies (for example, Monteiro *et al*., 2004; Bargues *et al*., 2006) further illuminate the ecological and geological context of the Triatominae extensive species radiation in the New World, and which in turn facilitates our understanding of their systematics.

POPULATION GENETICS

Population genetics has been an important tool in understanding the epidemiology of Chagas disease and in designing effective control methods to reduce local triatomine populations and interrupt transmission. Such studies have provided estimates of the level of genetic diversity present in populations, which are required to predict vector's adaptability (for example, domestication or development of pesticide resistance). Studies of gene flow among populations can help define the geographic coverage needed for successful control, the importance of sylvan populations in house infestation or the sources of re-infestation following insecticide treatment.

Genetic diversity

T. infestans and *R. prolixus* are two of the most medically important vectors, in large part because they are domestic species across most of their ranges. These two species generally show low genetic diversity in natural populations as well as in laboratory colonies, as determined by allozymes (Harry *et al*., 1992, 1993; Lopez and Moreno, 1995; Dujardin *et al*., 1998b; Monteiro *et al*., 2002) and confirmed by mtDNA and nuclear DNA sequence analyses (Table 1). By contrast, populations of *T. dimidiata* and *T. brasiliensis*, both of which are widespread across domestic, peri-domestic and sylvan habitats, generally show higher diversity, although nearby *T. dimidiata* populations in Costa Rica showed a single *ITS-2* haplotype (Table 1). Survival in these very diverse habitats, and movement among habitats, may have resulted in distinct selective pressures thus maintaining genetic diversity. Reduced genetic repertoire owing to founder effects during domestication could explain the low genetic diversity in *T. infestans* and *R. prolixus* (Schofield *et al*., 1999). However, domestication *per se* may not be sufficient to reduce genetic diversity as domestic populations showed nearly identical allozyme (Dujardin *et al*., 1987), or similar or even greater mtDNA diversity (Fitzpatrick *et al*., 2008; Piccinali *et al*., 2009) as nearby sylvan populations. Of course sylvan populations may represent feral derivatives of domestic populations (Dujardin *et al*., 1987). An alternative hypothesis is that low genetic variability reflects rapid and recent expansion owing to passive dispersal (Dujardin *et al*., 1998a).

A relatively higher genetic diversity in the *T. infestans* populations in the Bolivian Andes was used to propose this region as the origin of dispersal (based on allozymes (Dujardin *et al*., 1998a) and *cytb*

Table 1 DNA sequence population diversity of major triatomine species

Region	*Species*	*Marker*	*No. populations*	n	*Haplotype diversity* H_d	*References*
Mexico and Central America	*T. dimidiata*[a]	*cytb*	12	24	0.960	Dorn *et al*. (2009)
	T. dimidiata ss	*cytb*	7	58	0.901	Blandon-Naranjo *et al*. (2010)
	T. dimidiata[a]	*ITS-2*	93	190	0.918	Bargues *et al*. (2008); Dorn *et al*. (2009)
	T. dimidiata ss	*ITS-2*	7	58	0	Blandon-Naranjo *et al*. (2010)
South America	*T. infestans*	*cytb*	43	98	0.737	Monteiro *et al*. (1999); Giordano *et al*. (2005)
	R. prolixus	*cytb*	34	551	0.518	Fitzpatrick *et al*. (2008)
	T. brasiliensis ss	*cytb*	4	361	0.905	Almeida *et al*. (2008)
	T. brasiliensis[b]	*cytb*	17	136	0.920	Monteiro *et al*. (2004)
	T. infestans	*ITS-2*	31	35	0.591	Bargues *et al*. (2006)

Abbbreviation: ss, *sensu stricto*.
$H_d = n(1 - \Sigma \hat{x}_1^2)/(n-1)$, where n is the sample size and x is the frequency of a haplotype (Nei and Tajima, 1981).
[a]May include a cryptic species.
[b]Includes three proposed species: *T. brasiliensis/T. macromelasoma*, *T. juazeiro* and *T. melanica*.

sequence (Giordano *et al*., 2005)). This hypothesis is supported by cytogenetic differences in the Andean as compared with non-Andean *T. infestans*, DNA sequence differences (*ITS-2* (Bargues *et al*., 2006) and *cytb* (Monteiro *et al*., 1999 and Giordano *et al*., 2005)), as well as a highly significant F_{ST}=0.36 between populations from the two areas (based on *cytb* (Giordano *et al*., 2005)). Recent studies have shown considerable diversity in the Argentinean *T. infestans* populations as well (*cytochrome oxidase* sequence (Piccinali *et al*., 2009)). The speculation is that, perhaps multiple waves of immigration and rapid spread in Argentina, followed by genetic drift in isolated populations, has increased the genetic variability.

Genetic structure of populations

Vector migration is important for genetic mixing, re-infestation following pesticide treatment, the spread of genetically modified symbionts (Beard *et al*., 2002) and pesticide resistance genes. Although adults generally fly poorly (for example, Barbu *et al*., 2010), some species have been known to fly as far as 1 km, and wingless nymphs can walk tens of meters (Núñez, 1987). In addition to this active dispersion, passive dispersion by human activity, and perhaps carriage on animals and birds, is important over longer distances.

Over long distances, most studies show variation among populations consistent with the 'isolation by distance' model (Wright, 1943; Calderón *et al*., 2004; Pérez de Rosas *et al*., 2007, 2008; Piccinali *et al*., 2009; Table 2). At shorter distances, the picture differs among species and localities, and is often affected by pesticide treatment history.

T. infestans populations are generally highly structured as shown by allozyme analyses (Dujardin *et al*., 1987, 1998a; Brenière *et al*., 1998) and microsatellite markers (Table 2), as might be expected for a mostly domestic species. Interestingly, in areas where *T. infestans* has extensive sylvan populations, gene flow was also restricted among these sylvan populations and largely absent between the sylvan and domestic populations (Richer *et al*., 2007 and Table 2). Recent analysis of genomic (Perez de Rosas *et al*., 2011) and mtDNA (Torres-Perez *et al*., 2011) both support the hypothesis of two independent migration events of *T. infestans* in South America, and confirmed the existence of two distinct lineages.

By contrast, *T. dimidiata* populations show more extensive gene flow among houses within a village as well as among nearby villages in areas where it seasonally enters homes (Dumonteil *et al*., 2007; Table 2). Surprisingly, in areas apparently lacking sylvan populations, high levels of migration among houses within a village and nearby villages are inferred by high gene flow (Dorn *et al*., 2003; Stevens *et al*., unpublished data; Table 2). A finding of mostly unrelated or distantly related individuals within a house by random amplification of polymorphic DNA-PCR (RAPD-PCR) (Melgar *et al*., 2007) also supports substantial movement by *T. dimidiata*. *R. prolixus* also shows movement between most sylvan and domestic populations, indicating that sylvan populations can pose a risk for human infection (Fitzpatrick *et al*., 2008; Table 2).

INTERACTIONS VECTOR–PARASITE–HOST

The genetics and evolution of diseases vectors are influenced by relationships with their parasites (Schaub, 2006), hosts (Sacks and Kamhawi, 2001), symbionts (Dillon and Dillon, 2004) and with congeneric vectors (Pereira *et al*., 2006). These relationships are two-way interactions as vectors also have impacts on the other members of these 'vector-borne disease communities'. An integrated view of the significance of these interactions on the genetic and evolution of triatomines is largely unknown, but some bits of the puzzle are being pieced together.

Triatomine–*Trypanosoma* interactions

Once ingested during a blood meal, *T. cruzi* parasites undergo two major transformations in the vector. Trypomastigotes differentiate into the epimastigote stage, to multiply asexually, before differentiating into infectious metacyclic trypomastigotes (Figure 2). Parasite viability, development and multiplication are then influenced by physiological and biochemical interactions with the vector, according to its life stage and location inside the vector (see Azambuja *et al*., 2005b; Garcia *et al*., 2007, 2009).

Studies of the molecular interactions between triatomines and *T. cruzi* remain scarce as compared with other vector–parasite systems as they lie at the intersection between two neglected fields of research: (i) The immune system of hemipterans, less studied than in higher orders of insects (Ursic-Bedoya and Lowenberger, 2007), and (ii) the role of the innate immunity of insects in flagellate infections, less studied than the response to other microbial organisms (Boulanger *et al*., 2006). Most studies focus on mechanisms involved in the humoral response (but see Garcia *et al*., 2004 for insights into the cellular response), their genetic basis and, to a lesser extent, their effects on parasite life history

Table 2 Microsatellite subdivision among triatomine populations

Species	*No. of loci*	*No. insects per population*	*Geographic distance*	F_{ST}	*References*
Among villages					
T. dimidiata[a]	4	11–34	<280 km	0.0553	Dumonteil *et al*. (2007)
T. dimidiata	8	28–30	<13 km	0.066	Stevens *et al*., unpublished data
T. infestans	10	19–74	<1464 km	θ=0.135	Pérez de Rosas *et al*. (2007)
T. infestans	10	12–99	<31 km	0.018–0.192	Marcet *et al*. (2008)
T. infestans	10	18–70	<220 km	0.169	Pérez de Rosas *et al*. (2008)
T. infestans	10	1–78	<100 km	0.06	Pizarro *et al*. (2008)
Among ecotopes					
T. dimidiata[a]	4	18–41	Within villages, sylvan/domestic and peri-domestic	0.0096–0.0455	Dumonteil *et al*. (2007)
R. prolixus	9–10	10–39	Varied, sylvan/domestic	0.001–0.2	Fitzpatrick *et al*. (2008)
T. infestans	9	6–32	<1.1 km sylvan/sylvan; sylvan/domestic	0.0019–0.110; 0.026–0.072	Richer *et al*. (2007)

[a]May include a cryptic species.

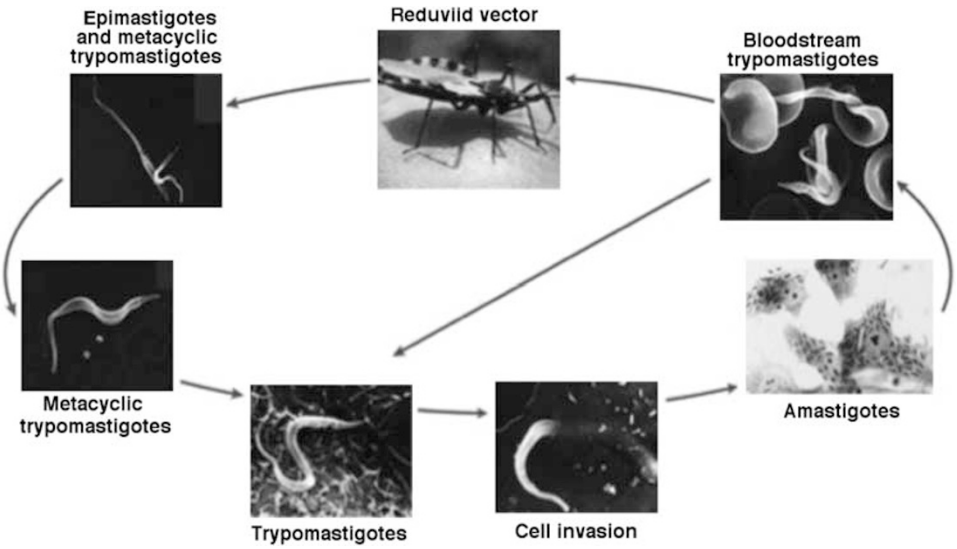

Figure 2 *T. cruzi* life cycle. Following a blood meal containing bloodstream trypomastigotes, *T. cruzi* parasites first differentiate into the epimastigote stage and multiply asexually, before differentiating into infectious metacyclic trypomastigotes. Upon entry of trypomastigotes in the mammalian host, those are able to invade cells and further replicate as intracellular amastigotes (from Andrade and Andrews, 2005, with permission).

and within-host dynamics (Table 3). For example, lectins and antimicrobial peptides such as defensins and lysozymes have been described in several triatomine species (Table 3). Many of the corresponding genes are mostly expressed in the stomach (but see Lopez *et al*., 2003), where symbionts grow intensively after blood meals (Araújo *et al*., 2006; Balczun *et al*., 2008). Importantly, defensin is strongly upregulated in the mid-gut of *T. brasiliensis* following infection with *T. cruzi* (Waniek *et al*., 2011). Lysozyme genes are expressed in both the fat body and the mid-gut of triatomines. Trypanolytic factors, the prophenoloxidase cascade and the impact of various oxides have also been studied, but almost exclusively in the hemocel of *R. prolixus* infected by *Trypanosoma rangeli*.

One of the most intriguing outcomes of triatomines–*Trypanosoma* interactions is that closely related parasites have very different transmission modes. *T. rangeli* spreads from the gut to the hemocoel (Mello *et al*., 1995) and, at least for some (KP1−) populations, to the salivary gland of *Rhodnius* species, and is transmitted by bites (Guhl and Vallejo, 2003). By contrast, *T. cruzi* infection is limited to the gut, so that it is transmitted through the vector feces. It is still unclear whether such a difference results from (1) an adaptation of *T. cruzi* to limit its spread from the gut and avoid the insect immune response primarily located in the hemocoel (Whitten *et al*., 2001; Lopez *et al*., 2003), or (2) an adaptation of *T. rangeli* to reduce the impact of the humoral response of the insect, for example, by infecting hemocytes and inhibiting the proPo pathway that leads to melanization (Gregorio and Ratcliffe, 1991; Gomes *et al*., 1999; Garcia *et al*., 2007). Interestingly, whereas the first hypothesis involves a trade-off between an increase in *T. cruzi* growth rate within its vector and a reduced transmission to mammalian hosts, the second hypothesis involves an increase in *T. rangeli* development rate in the hemolymph, with no detrimental impact on transmission.

There is also evidence that, according to variations in their immune response, vectors are likely to be biological filters transmitting differently lineages/genotypes of *T. cruzi* and *T. rangeli* (Vallejo *et al*., 2009). Lectins produced in the stomach of *R. prolixus* agglutinate Dm28 clones but not the Y-strain of *T. cruzi* (Mello *et al*., 1996; Ratcliffe *et al*., 1996). The presence of trypanolytic factors in the *R. prolixus* hemolymph impedes the development of *T. rangeli* KP1− but not the development of *T. rangeli* KP1+ (Vallejo *et al*., 2002, 2003).

The above mechanisms and outcomes of vector–parasite interactions can be thought of in terms of co-evolutionary adaptations, especially within the *Rhodnius* genus, where *T. rangeli* is known to be pathogenic to its vector, and where parasite/vector association appear to be more specific (Vallejo *et al*., 2009). By contrast, a common view is that *T. cruzi* has little impact on its vector's life history (Zeledón, 1981; Schaub, 1989, 2006; Vallejo *et al*., 2009), and thus produces no or little selective pressure on its vector. Such a view is supported by a lack of change in developmental time (Zeledón, 1981; Schaub, 1988), life span and fertility (Zeledón, 1981; Schaub, 1989), and feeding behavior (Schaub, 2006; Garcia *et al*., 2007) when triatomines are infected by *T. cruzi* in the laboratory. But there is counter-evidence (Schaub, 1989, 1994; Botto-Mahan *et al*., 2006; Botto-Mahan, 2009; Nouvellet *et al*., 2011) and laboratory experiments often suffer biases, thus weakening their applicability to natural populations (Schaub, 1989). Typically, natural conditions involve long periods of starvation potentially leading to competition for rare nutrients between a vector and its parasites (Kollien and Schaub, 2000). In fact, resistance to starvation decreases after *T. cruzi* infection in both *T. dimidiata* (Vargas and Zeledon, 1985) and *T. infestans* (Schaub and Lösch, 1989). Nonetheless, as *T. cruzi* develops in the gut rather than in the hemolymph, it is thought to interfere less with the vector than *T. rangeli* (Lopez *et al*., 2003; Ursic-Bedoya *et al*., 2008). However, the mid-gut (and not only the hemocoel) of triatomines has been shown to be immune-reactive, and (artificial) infections of the hemolymph by *T. cruzi* do induce an immune response (Ursic-Bedoya and Lowenberger, 2007). As *T. cruzi* is able to enter the hemocoel (Zeledon, 1987; Mello *et al*., 1995), 'mid-gut habitat preference' observed for *T. cruzi*, could readily be the outcome of a (potentially costly) immune process preventing the invasion of the hemocoel (Whitten *et al*., 2001). A detailed understanding of the mechanism blocking the transit of *T. cruzi* across the mid-gut is thus critically needed to convincingly evaluate the actual cost of *T. cruzi* infection to the vector.

Overall, although studies of the molecular aspects of the vector immune responses are developing, they still lack quantitative assess-

Table 3 Components of the humoral response of triatomines

	Location[a]	*Effect*[b]	*Triatoma*	*Pathogens*[c]	*References*
Lectins	HEM	–	*P. megistus*	–	Gomes *et al*. (1988)
	CR	–	*T. infestans*	–	Grubhoffer *et al*. (1997)
	–	Agglutination	*R. prolixus*	*T. cruzi*	Mello *et al*. (1996)
	CR	–	*R. prolixus*	*T. cruzi/T. rangeli*	Ratcliffe *et al*. (1996)
	ST+INT+HEM	–	*R. prolixus*	*T. cruzi*	Pereira *et al*. (1981)
	HEM	–	*R. prolixus*	*T. rangeli*	Mello *et al*. (1999)
Antimicrobial peptides					
Defensin	FB (+Int)	No*	*R. prolixus*	*Bacteria*	Lopez *et al*. (2003)
	ST	–	*T. brasiliensis*	–	Araújo *et al*. (2006)
	SG	–	*T. infestans*	–	Assumpção *et al*. (2008)
	ST	–	*T. brasiliensis*	–	Waniek *et al*. (2009)
Lysozymes	(+FB+SI+SG)	–	*R. prolixus*	*T. cruzi/T. rangeli*	Mello *et al*. (1995)
	HEM	–	*T. infestans*	–	Kollien *et al*. (2003)
	ST	–	*R. prolixus*	*T. rangeli*	Gomes *et al*. (1999)
	–	–	*T. brasiliensis*	–	Araujo *et al*. (2006)
	ST	No*	*T. infestans*	–	Balczun *et al*. (2008)
	SmI		*R. prolixus*	*T. cruzi*	Ursic-Bedoya *et al*. (2008)
	Mid-gut+FB				
Trypanolytic factors	HEM		*R. prolixus*	*T. rangeli*	Mello *et al*. (1995)
	HEM	↓Develop[t]	*R. prolixus*	*T. rangeli KP1–*	Pulido *et al*. (2008)
	HEM	No effect	*R. prolixus*	*T. rangeli KP1+*	Azambuja *et al*. (2004); Pulido *et al*. (2008)
Prophenoloxidase cascade (melanization+production of highly reactive and toxic compounds)	HEM	–	*R. prolixus*	*T. rangeli*	Mello *et al*. (1995)
	HEM+FB	–	*R. prolixus*	*T. rangeli*	Gomes *et al*. (1999)
	FB	–	*R. prolixus*	*T. rangeli*	Gomes *et al*. (2003)
	HEM	↓ Survival	*R. prolixus*	*T. rangeli*	Garcia *et al*. (2004)
	SmI+FB	–	*R. prolixus*	*T. cruzi*	Ursic-Bedoya and Lowenberger (2007)
Nitric oxide (nitrite and nitrate) and superoxide	HEM	↓ Survival	*R. prolixus*	*T. rangeli*	Whitten *et al*. (2001) (2007)

[a]The humoral response has mostly been studied in the hemocoel (HEM) and in the fat body (FB), where it is known to develop in insects. Components of the humoral response have also been studied in the fore-gut, specifically in its last part, the crop (CR), in the mid-gut (both in its anterior, non-digestive part (the cardia (CA) and stomach (ST)) and in its posterior, digestive part (the small intestine (SmI)).
[b]Most studies do not test the effect of the molecular mechanism on the parasite life history (denoted by '–'), or speculate that the effect should be minor or null ('No*'). Still, a few experiments have investigated the effects on parasites, which are then specified in the table.
[c]Some studies include an immunological challenge by a *Trypanosoma* or bacteria, and this is then specified in the table; others do not include such challenge (denoted by '–').

ments of their effect on parasite life history. Such measurements are critically needed to further understand the mechanisms as well as the evolution of these interactions; especially, the assessment of the effects of *T. cruzi* under biologically realistic conditions, that is, when infection is considered in synergy with other stress factors such as starvation or co-infection with additional strains. Quantitative assessments would make key contributions to the understanding of the interactions between triatomines and trypanosomes, and ultimately the transmission of Chagas disease.

Triatomine–*Trypanosoma*–microbiota interactions

Experimental studies indicate that a community of intestinal microorganisms might regulate the development of parasites in the insect mid-gut (and thus vector competence), although the underlying mechanisms remain largely unknown (Azambuja *et al*., 2005a). Numerous species of bacteria inhabit the gut of triatomines (listed in Vallejo *et al*., 2009) and they typically undergo massive population size expansion after a blood meal (10^2- to 10^4-fold increases; Azambuja *et al*., 2004), whereas trypanosomes sharply decrease in number (Azambuja *et al*., 2005a). The trypanolytic activity of two strains of *Serratia marcescens* (SM365 and RPH) has been shown recently to be associated with its attachment to the membrane of the *T. cruzi* Y-strain (Azambuja *et al*., 2004). The rapid bacterial expansion could then contribute to decreasing parasite abundance in a direct manner. Indirect effects are also expected as increase of bacterial populations activates the production of lysozyme (Kollien *et al*., 2003) and defensin (Araújo *et al*., 2006), which are likely to affect the multiplication of trypanosomes. Furthermore, intestinal microorganisms have significant effects on the vectors. Typically, asymbiotic triatomines suffer important developmental delays, and increased mortality (Eichler and Schaub, 2002; Vallejo *et al*., 2009). A potential explanation is that triatomine blood meals lack essential vitamins and nutrients, which are supplied by symbionts (Beard *et al*., 2002). Microorganisms may thus influence the vector–parasite interaction through their relationships with both triatomines and trypanosomes. It has indeed been hypothesized that *R. prolixus* has evolved a lower antimicrobial peptide production in the intestine to allow essential symbionts to develop. This could have induced the evolution of a mid-

gut habitat for *T. cruzi* (Ursic-Bedoya *et al*., 2008), leading to the relatively inefficient mode of transmission by feces, with key implications for the triatomine–trypanosome interaction.

Triatomine species interaction

Studies of interaction between triatomines are rare, and focus on the effect of *T. infestans* on other Triatominae. *T. infestans* is thought to have competitively displaced *P. megistus*, *T. sordida*, *T. brasiliensis* and *T. pseudomaculata* from different parts of South America (Figure 1) (Pereira *et al*., 2006). Such ecological displacement has indeed been observed in laboratory experiments where *T. infestans* consistently excluded *T. sordida* after a few months (Bar *et al*., 1994; Oscherov *et al*., 2001, 2004). Indirect field evidence of competition includes observations of higher house infestation and abundance of *T. sordida* (Canale *et al*., 2000; Oscherov *et al*., 2004), *P. megistus* (Villela *et al*., 2005) and *T. brasiliensis* (Pereira *et al*., 2006) after removal of *T. infestans* by control programs. Intraspecific and interspecific competition for blood meals appears to be the key mechanisms to explain such exclusion. In mixed experimental populations, the *T. sordida* blood-meal size is reduced, leading to lower larval survival rates and reduced population growth, whereas *T. infestans* remains unaffected. Similarly, the competitive advantage of *T. infestans* could result from its ability to ingest larger blood meals, while having a lower requirement to complete its (shorter) life cycle (Pereira *et al*., 2006). Clearly, interactions between (domestic) *T. infestans* and (peri-domestic and sylvatic) other triatomine species also involve a dispersal-competitive trade-off (Oscherov *et al*., 2004), and could thus be better understood if studied in a meta-population dynamic framework such as the ones available for triatomines (Gourbière *et al*., 2008; Barbu *et al*., 2009, 2010, 2011; Slimi *et al*., 2009, and references therein). Understanding the balance between competitive and dispersal abilities will allow understanding the impact of competition between triatomines on parasite transmission, as suggested by theoretical studies (Gourbière and Gourbière, 2002; Begon, 2008).

The interaction between triatomines, their symbionts and trypanosomes establishes a community network with many potential positive and negative feedbacks (Figure 3). In this context, the genetics and evolution of triatomines are likely to follow non-trivial dynamics. Better knowledge of this complex dynamic will surely be gained by studying the *R. prolixus–T. rangeli* and *T. infestans–T. cruzi* interactions, two systems with interesting transmission differences, and by looking at the influence of major triatomine life history on pathogen dynamics (Menu *et al*., 2010). Additionally, studies of other important vectors of *T. cruzi* and Chagas disease will be needed to reach the general level of understanding required to transfer basic knowledge into safe and efficient control strategies.

VECTOR CONTROL: BEYOND MASSIVE INSECTICIDE SPRAYING

Most studies of triatomines have been motivated by the need for vector control interventions to prevent Chagas disease transmission to humans. The general aims were thus to identify vector species present in human dwellings, to understand the success or failure of indoor insecticide spraying, as well as to design more rational and evidence-based vector control interventions.

Because the medical entomological importance of vectors was traditionally assumed to be associated with the species, efforts have focused on taxonomic identification, for example, resolving the issue of the sylvatic dark morph of *T. infestans* (Noireau *et al*., 1997) and or evaluating gene flow between sylvatic and domestic *R. robustus* and *R. prolixus* in Venezuela, to show that *R. robustus* and *R. prolixus* are distinct species (with rare introgression), and implicating the sylvatic *R. prolixus* populations as potential contributors to human transmission.

T. dimidiata phenotypic variations have also puzzled entomologists for many years, the more so because it was found in a wide variety of habitats, and presented widely different levels of adaptations to human dwellings, ranging from highly domiciliated to preferentially sylvatic (Dorn *et al*., 2007). The existence of a species complex is now emerging, supported by molecular, morphometric and cytogenetic data (Bustamante *et al*., 2004; Lehmann *et al*., 2005; Panzera *et al*., 2006; Bargues *et al*., 2008, Dorn *et al*., 2009), but the association of the different species/subspecies with particular levels of adaptation to the domiciles and vectorial competence is still unclear. For example, two of the distinct *T. dimidiata* cryptic species behave in an identical non-domiciliated manner in the Yucatan peninsula, although one was thought to be much more domiciliated (Dorn *et al*., 2009; Herrera-

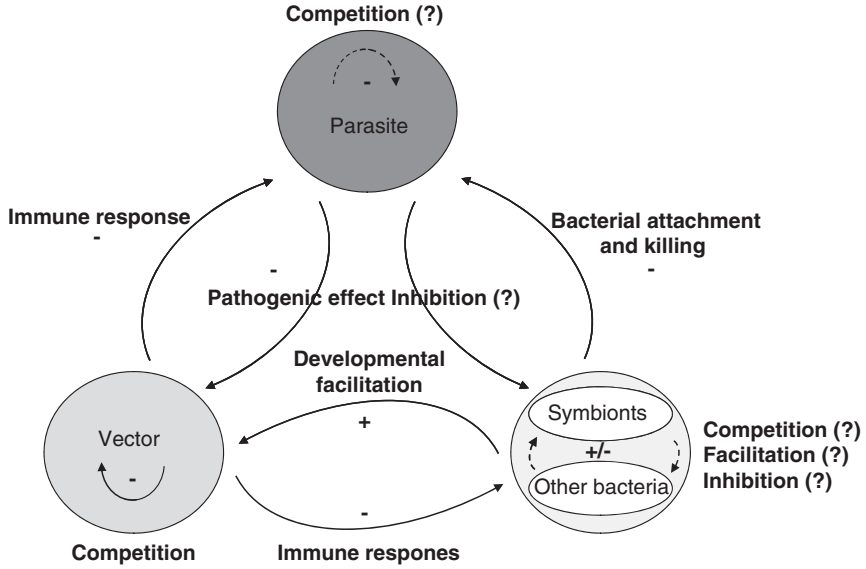

Figure 3 Interaction network. *Triatoma*, *Trypanosoma* and microorganisms (either symbiotic or not) interact with one another. Direct or indirect interactions, which have been documented in this review, are all included in this figure. The question marks indicate interactions, which have not yet been investigated.

Aguilar *et al*., 2009). This suggests that domiciliation may rather be associated with ecological environments than with species status, which emphasizes the need for more detailed population genetic studies to identify the relevant vector populations.

Indeed, population genetics has been a key tool to define panmictic populations and dispersal range, and thus explain outcomes of vector control efforts. Control strategies based on indoor insecticide spraying began to be tested in the field in the 1940s, and national programs were initiated in the 1970s in several countries such as Brazil, Argentina, Venezuela and Chile, and culminated with several international initiatives such as the Southern Cone or the Central American initiatives in the 1990s (Schofield and Dias, 1999; Dias *et al*., 2002).

The success of such interventions, which resulted in a >90% reduction of house infestation by triatomines and a similar reduction in vectorial transmission of *T. cruzi* to humans in most of the southern cone region (Dias *et al*., 2002; Schofield *et al*., 2006), relied mostly on the marked genetic structure of *T. infestans* populations and the lack of gene flow among them. However, the elimination of *T. infestans* from houses opened the way for secondary species to start invading this habitat, but again, little is known about triatomine inter-species competition.

As expected, a reduction in the genetic diversity of triatomine populations has been observed following pesticide application, presumably as a result of severe bottlenecks (Garcia *et al*., 2003). However, some studies have reported an unchanged or even greater genetic diversity after insecticide spraying (Pérez de Rosas *et al*., 2007, 2008). The authors speculate that the split of a population into several ones, each with different combination of alleles, followed by genetic drift and population expansion could retain the diversity. The effects of anthropogenic changes such as deforestation and global climate change are yet unknown, although the Amazon is becoming a new area for Chagas transmission, coincident with massive deforestation (Diotaiuti, 2009).

In the Gran Chaco region (which encompasses Northern Argentina and parts of Bolivia and Paraguay; Figure 1), vector control measures have notoriously failed. Insects present some months after pesticide treatment are nearly always survivors or migrants from nearby peri-domestic populations as revealed by allozyme, mtDNA and microsatellite analyses (Dujardin *et al*., 1996; Garcia *et al*., 2003; Perez de Rosas *et al*., 2007; Pizarro *et al*., 2008). Heterogeneity in peri-domestic environments (from wood piles to granaries to animal corrals) makes control very difficult (Zu Dohna *et al*., 2007). In addition, pesticide resistance has now been detected in several of these populations (Gonzalez Audino *et al*., 2004; Picollo *et al*., 2005; Toloza *et al*., 2008, Germano *et al*., 2010a; Lardeux *et al*., 2010). The molecular mechanisms of resistance evolved by triatomines may rely on various detoxification pathways (Orihuela *et al*., 2008). Recent crossing experiments indicate that deltamethrin resistance inheritance is autosomal and semi-dominant, and seems to involve up to three different genes (Cardozo *et al*., 2010; Germano *et al*., 2010b). However, more studies are required to further understand the development of insecticide resistance in these species.

When triatomine populations are present in domestic, peri-domestic and sylvatic ecotopes, and gene flow among ecotopes is significant, insecticide spraying is of limited efficacy (Dumonteil *et al*., 2004; Barbu *et al*., 2009), likely because populations can be rapidly rebuilt by migrants. Thus, the control of such non-domiciliated or autochthonous triatomines remains a major problem for vector control (Guhl *et al*., 2009), and novel approaches such as screens, bednets and house improvements will be necessary to interrupt transmission (Figure 4) (Monroy *et al*., 1998, 2009; Barbu *et al*., 2009, 2011; Ferral *et al*., 2010).

Another potential alternative is to manipulate the vector–parasite interaction and modified vector symbionts may allow reducing the parasite transmission rate. For example, *Rhodococcus rhodnii* can be transformed so that it produces peptides of *Lepidoptera* (Beard *et al*., 1998; Beard *et al*., 2002) or a mammalian antibody (Durvasula *et al*., 1999) that strongly reduces the number of *T. cruzi* parasites in the vector. This parasite control strategy has been referred to as paratransgenesis, and may provide new options for Chagas disease control.

Overall, much integration is still needed to take advantage of the genetic and molecular knowledge available to move from the empirical insecticide spraying strategies to the design of more rational and evidence-based control interventions against triatomines. The examples detailed above illustrate how vector control can benefit from genetic studies, and point to gaps in our current understanding of some of these aspects.

CONCLUDING REMARKS

An increasing number of genetic studies have contributed significantly to our understanding of triatomine biology, from species identification to phylogeny and phylogeography and population genetics. However, a major drawback of many of these studies is that they do not address aspects of the vectorial capacity of the different populations/species (Mas-Coma and Bargues, 2009), making interpretation

Figure 4 Innovative vector control interventions. Novel interventions, based on detailed knowledge of triatomine species population dynamics and genetics, may rely on improving wall plastering of houses using traditional methods, which prevents colonization (**a**), or on the installation of insect screens on windows, to prevent transient infestation (**b**).

of their results in terms of parasite transmission and epidemiological relevance very speculative. Integrative studies including aspects such as *T. cruzi* infection rates, blood meal analysis or vector–parasite interactions with population genetic data are thus critically needed to clearly understand the links between population structure and parasite transmission. Such studies would also allow a better understanding of major processes such as the transition from predatory to hematophagous bugs, the domiciliation of triatomines or the impact of control interventions on vectorial transmission.

Whereas several microsatellite markers have been identified in major triatomine species such as *T. infestans*, *T. dimidiata* or *R. prolixus*, molecular markers are lacking for many other species. Also, genome-wide single-nucleotide polymorphism genotyping approaches, which are now popular in other insect vector species (Megy *et al.*, 2009; Horton *et al.*, 2010; Li *et al.*, 2010), have not yet been developed in triatomines. In that respect, the ongoing sequencing of the *R. prolixus* genome (Megy *et al.*, 2009) will provide a very valuable tool to further understand triatomine biology and genetics. Nonetheless, additional triatomine genomes will be required, particularly from the *Triatoma* genus, and major vector species such as *T. infestans* or *T. dimidiata* would be excellent candidates for future genome sequencing. Triatomine genomes would also open the way to extensive studies of vector–parasite interactions at the molecular level, an area where little is known, in spite of the uniqueness of the mechanism of *T. cruzi* transmission through insect feces. Thus, although triatomine genetics research has lagged somewhat compared with other insect species, these species represent unique models for key biological processes, and we can expect that several novel approaches will be developed in the near future to expand our understanding of *T. cruzi* vectorial transmission, vector–parasite interactions and the development of rational vector control strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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