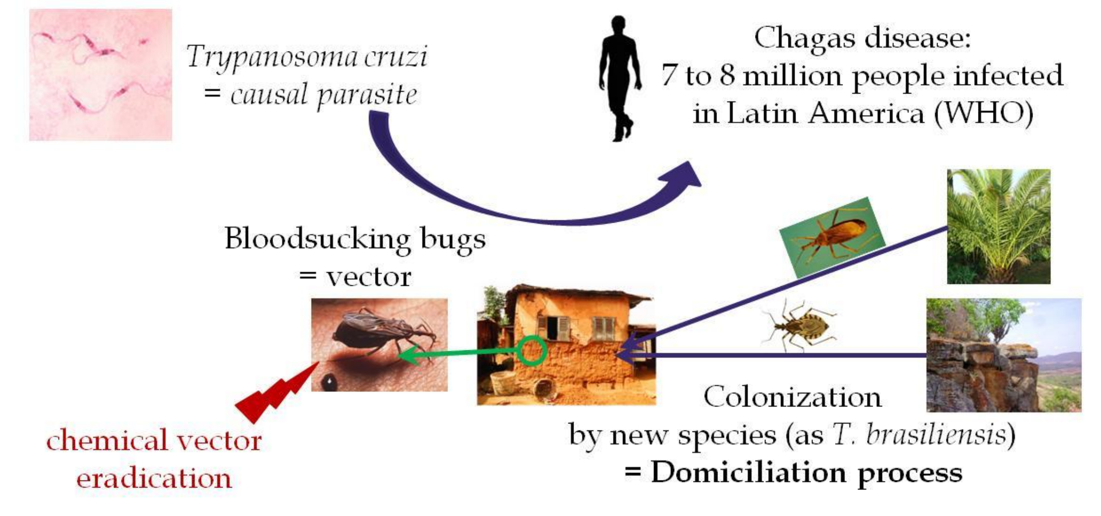
Chemosensory transcriptomes of bloodsucking bugs, Chagas disease vectors

AIM → Search for genes implicated in the domiciliation process of *Triatoma brasiliensis* using differential gene expression analysis

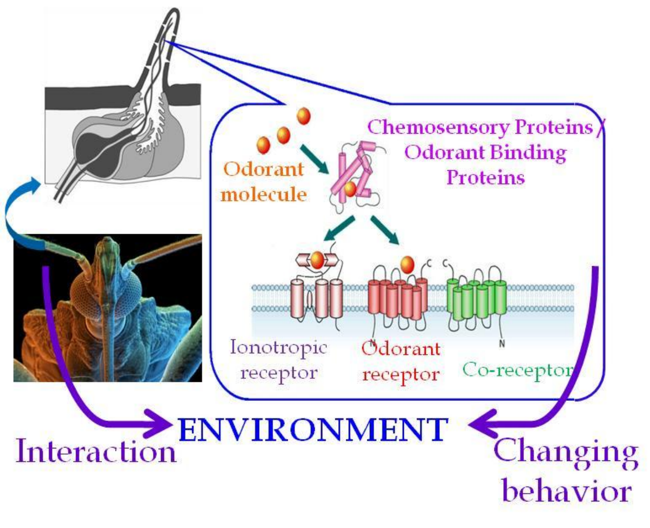
INTRODUCTION

Chagas disease vectors and domiciliation process



- → Need to understand the domiciliation process to improve vector control and knowledge about the adaptation of species to anthropogenic systems
- → Search for genes involved in this process

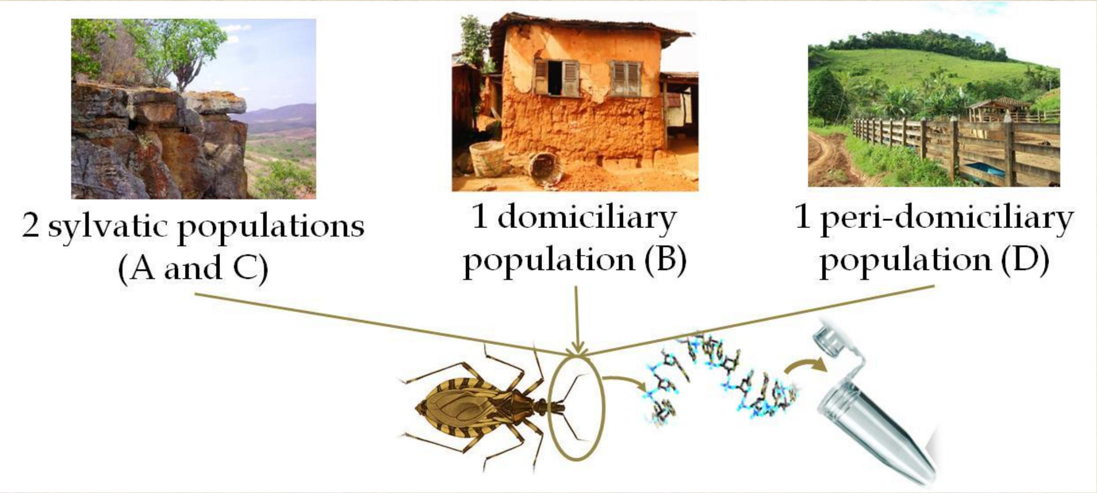
Chemosensory system = candidate genes for domiciliation process



Bugs interact with their environment and behave thanks to their chemosensory system. It could play an important role in anthropogenic adaptation. Understanding the chemosensory system of Triatominae could shade light on the domiciliation process

MATERIALS & METHODS

RNA extraction Triatoma brasiliensis and sequencing



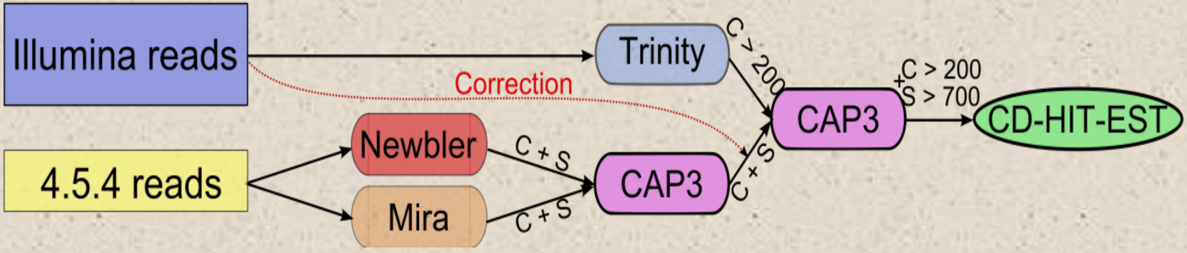
→ RNA extraction from antennae and rostrum
→ 454 and Illumina Sequencing

De novo *assembly of the T. brasiliensis reference transcriptome*

In comparative studies of non-model organisms, the conclusions depend on the quality of the assembly. It's fundamental to draw an assembly as optimized as possible from an available dataset. We tested several assembly methods from different datasets of T. *brasiliensis*:

Samples	Sequencing methods	Software
Merged sample → 1 sample from several individuals from several populations and sexes → 454 sequencing : 555,854 reads	454	MIRA
		Newbler
		MIRA + Newbler + CAP3
Population samples (4 populations, 2 sexes) → Several individuals of the same sex and from the same population per sample →Sequenced in Illumina single reads: 5 millions to 53 million reads per sample	Illumina single reads	Trinity
		Trans-Abyss
		Oases
Individual sample 1 sample from a unique sylvatic female → Brain added to RNA extraction → Sequenced in Illumina paired-end: 46 millions X2 reads	Illumina paired-end	Trinity
Pool of Merged sample and Individual sample	454 + Illumina paired-end	MIRA + Newbler + CAP3 / Trinity
	454 **Corrected** + Illumina paired-end	

We compared theses tests using several criteria (contigs number, N50, completeness and presence of potential chimeric contigs). Finely, we selected the last assembly pooling individual sample sequenced in Illumina paired-end and merged samples sequenced in 454. Homopolymers repetitions errors were corrected mapping Illumina reads in 454 contigs and correcting polymorphism following Illumina reads.



De novo assembly workflow, Marchant et al., 2014

Differential expression analysis

-Mapping each sample on *T.brasiliensis* reference transcriptome (BWA)
-Differential expression analysis with DESeq2 → comparison of environmental conditions
-Annotation of differentially expressed transcripts with blastx against swissprot database

RESULTS

Reference transcriptome results (Marchant et al., 2014)

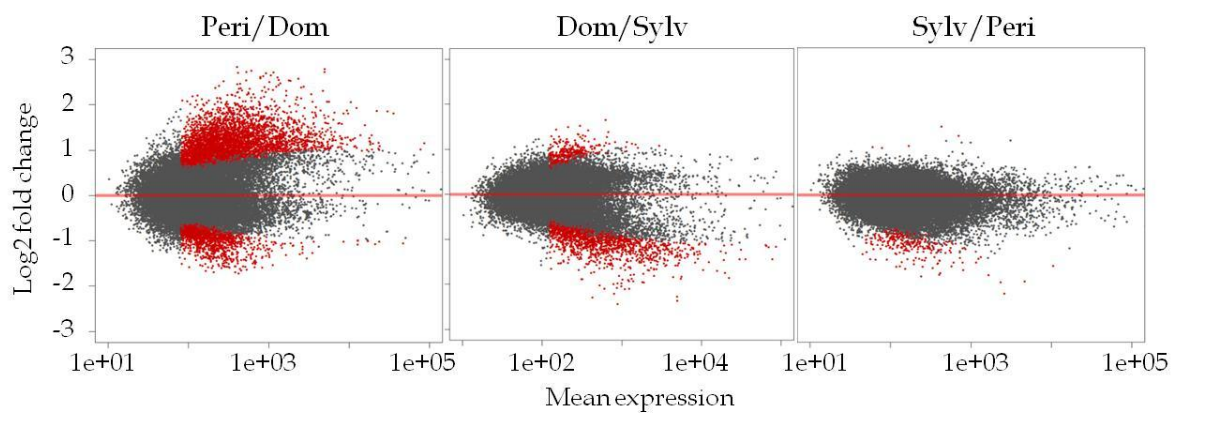
Contig number	N50	Length distribution		Completness
		mean	**total length**	
42,293	1146 bp	1110 bp	46,952,869 bp	88,71 %

Differential expression analysis results

Peri/Dom	Dom/Sylv	Sylv/Peri
4597	1162	228

Number of differentially expressed genes between 3 environments (Padj < 0.05)

<u>Differentially expressed profiles between different environments</u>

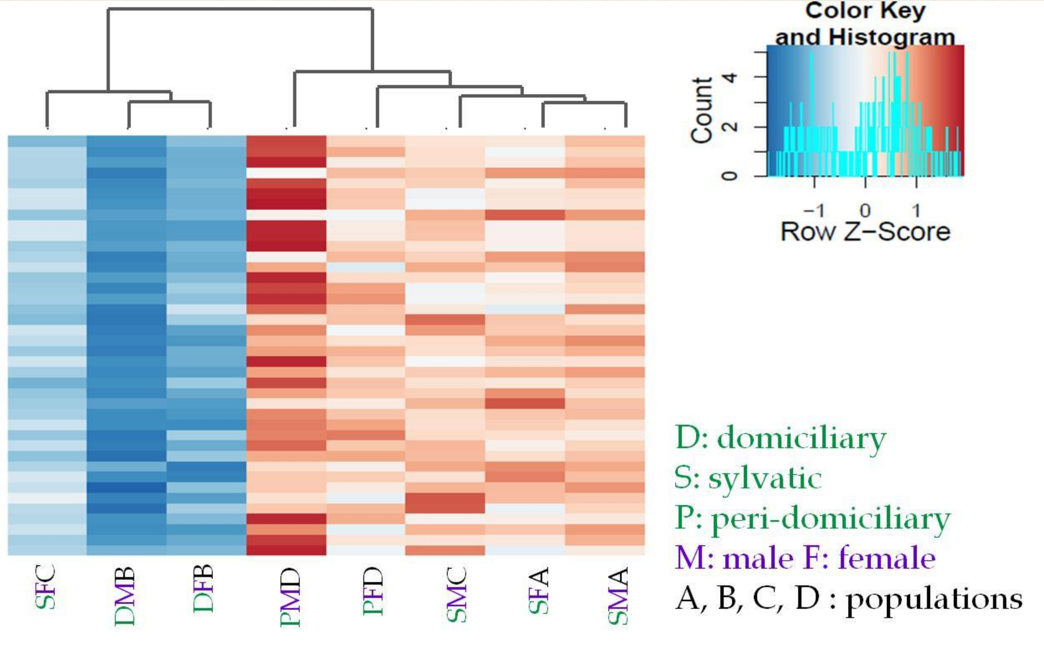


Log2 fold change: Attributable to a variable over the mean of normalized counts
Red points: genes differentially expressed (Padj < 0.05)

<u>Among top 50 differentially expressed genes of environment comparisons, a majority of chemosensory genes</u>

-**Odorant-binding proteins:** transport odorant molecules to olfactory receptors in sensilla
-**Pheromone-binding proteins:** transport pheromones to olfactory receptors
-**Cytochrome P450**: have been proposed to act as odorant-degrading enzymes in several insect species
-**Takeout**: novel molecular link between circadian rhythms and feeding behavior

<u>Genes clustering based on differentially expressed OBP or P450 genes (under-expressed in domiciliary samples)</u>



CONCLUSIONS

- ***De novo* assembly**: merging of long reads (454 for example) and short reads (Illumina paired-end) from a single individual
- **Domiciled bugs** have gene expression profiles that differ from **sylvatic and peri-domiciled**
- Differences in gene expression are also found between **males and females**
- As expected, genes differentially expressed were found in **the chemosensory system** between samples from different environments
  + Takeout : involved in the feeding behavior of adult response to the diet, the circadian rhythm

Perspectives:

→Differential expression analysis with other pipeline (including edge R) and comparison with DESeq2 results

→These results need to be **confirmed by qPCR.**

Valorization

Paper:

Marchant, A., Mougel, F., Almeida, C., Jacquin-Joly, E., Costa, J., Harry, M., 2014. *De novo* transcriptome assembly for a non-model species, the blood-sucking bug *Triatoma brasiliensis*, a vector of Chagas disease. Genetica 1–15. doi:10.1007/s10709-014-9790-5

Meetings:

Axelle Marchant1, Nicolas Glaser, Florence Mougel , Emmanuelle Jacquin-Joly, Myriam Harry 2014 Contribution of the chemosensory system to insects adaptation to anthroposystems: transcriptomic studies in *Sesamia nonagrioides* and in two bugs vectors of Chagas disease. HTS network meeting BASC "Adaptation of eukaryotes to environmental changes", 3 April 2014. Talk

A. Marchant, F. Mougel, C. Almeida, E. Jacquin-Joly, J. Costa, M. Harry 2014 *De novo* transcriptome assembly for a non model species, the blood-sucking bug *Triatoma brasiliensis*, a vector of Chagas disease. European meeting: Bioinformatics for Environmental Genomics, Lyon, 28th May. Talk

A. Marchant, F. Mougel, C. Almeida, J. Costa, E. Jacquin-Joly, M. Harry 2014 Chemosensory transcriptoms of bloodsucking bugs, Chagas disease vectors. Seventh International Symposium on Molecular Insect Science, Amsterdam, 13-16 July 2014. Poster presentation

A. Marchant, F. Mougel, C. Almeida, J. Costa, E. Jacquin-Joly, M. Harry 2014 Study of the chemosensory transcriptome of bloodsucking bugs, vectors of Chagas disease. Petit pois déridé, Orsay, 25-28 July 2014. Talk

A. Marchant, F. Mougel, C. Almeida, J. Costa, E. Jacquin-Joly, M. Harry 2014 Comparative Transcriptomics in Chagas disease vectors: a focus on chemosensory genes. Joint 2014 Annual Meeting British Ecological Society and Société Française d'Ecologie 9 – 12 December, Grand Palais, Lille, France. Talk