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Comparative Genome-Wide Analysis of Mirror Repeats in Virulence-Associated Genes of *Leishmania Donovani* and *Trypanosoma Cruzi*

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Abstract: *Mirror repeats (MRs) are symmetrical DNA sequences that can form non-B DNA structure in the genome affecting aspects of genome stability, gene regulation, recombination and evolutionary processes. For kinetoplastid parasites, repetitive DNA plays a role in virulence, antigenic variation, and genomic plasticity, but comparative studies of the mirror repeats are limited. The current study is a computational study of all the virulence associated genes of Leishmania donovani and Trypanosoma cruzi using miRNA mapping approach. Genomic sequences were downloaded from public databases to check for the presence of mirrorepeats, abundance of mirrorepeats, length, GC content, chromosomal distribution and association with the key virulence genes by using bioinformatics tools. Analysis showed that both parasites have a lot of mirror repeats, and that T. cruzi is more abundant due to the large and highly repetitive genome. A few genes coding GP63, amastins, trans-sialidases, mucins and cruzipain were often harboring mirror repeats. The results indicate that the presence of repeats in the mirror orientation could have a role in genome evolution, adaptive virulence and pathogenicity of the parasites, and might serve as markers for comparative genomic studies and molecular diagnostics.*

Keywords: Mirror repeats, Comparative genomics, Leishmania donovani, Trypanosoma cruzi, Virulence genes, Genome analysis, Repetitive DNA, Bioinformatics

I. INTRODUCTION

Protozoan parasites continue to be an important public health problem, especially in the tropics and subtropics, where neglected tropical diseases are endemic. Two of the three families, Leishmania and Trypanosomatidae, are responsible for visceral leishmaniasis, caused by Leishmania donovani, and Chagas disease, caused by Trypanosoma cruzi. These parasites have an extremely dynamic genome, where polycistronic transcription, genome-wide gene duplication, the presence of a large proportion of repetitive DNA and highly plastic genome are all features that allow quick adaptation to various host environments and play a role in parasite virulence.

Repetitive DNA elements include unique symmetrical sequences, known as mirror repeats (MRs), which can lead to the formation of non-B DNA structures, such as triplex (H-DNA), that play a role in genome stability, evolution, and DNA replication, transcription, recombination. While there have been reports of chromosomal rearrangements and gene regulation associated with mirror repeats in higher organisms, the distribution and roles of mirror repeats in kinetoplastid parasites are not well known. The genome studies that have been conducted so far have mainly focused on genes that are part of families associated with virulence, antigenic variation and chromosome copy-number variation, and comparatively little attention has been paid to structural repetitive elements like mirror repeats.

A comparative genome-wide computational study of virulence-associated gene (VAG) mirror repeats of Leishmania donovani and Trypanosoma cruzi is presented. The investigation involves the comparison of the abundance, distribution, GC content, length of the repeat, and chromosomal localization of the mirror repeats using publicly

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available genomic sequences and bioinformatics tools. The results are useful for understanding the function of mirror repeats in genome organization, parasite evolution and pathogenicity and are also useful as molecular markers or as targets for new pathogen diagnostics, therapeutics and comparative genomic studies.[1]

II. LITERATURE REVIEW

Black et al. (2023) examined the mechanism of how genome plasticity can contribute to the ability of Leishmania to adapt and evolve and concluded that repetitive DNA, chromosome instability, aneuploidy and copy-number variation play a major role in parasite adaptation and evolution. The authors said these genomic changes allow the parasite to withstand environmental stress, and evade host immune defenses while developing resistance to drugs while remaining pathogenic. The review also emphasized on the significance of structural genomic variation in virulence and adaptation. These results corroborate the present study, and suggest that repetitive DNA sequences such as mirror repeats may be important for genome organization, evolutionary flexibility and survival of parasites.[2]

Guiblet et al. (2021) explored the biological relevance of non-B DNA structures and showed that the presence of mirror repeats can lead to alternative DNA structures, such as triplex (H-DNA). These structures affect DNA replication, polymerase movement, mutation rate, genome stability and are associated with genomic variations and rearrangements, it was reported. The authors highlighted that non-B DNA motifs are of crucial importance in genome organization and evolutionary processes. They conclude that their observations offer a solid theoretical basis for the study of mirror repeats as potentially significant functional units of repetitive DNA that can play a role in genome plasticity, virulence and adaptive evolution of kinetoplastid parasites.[3]

Franssen et al. (2020) carried out a genome-wide analysis of the species. The study found that the structural genomic variation, such as variation in chromosome copy numbers, in gene amplification and in sequence diversity, is important for the survival of parasites, adaptation to hosts and virulence. The authors emphasize genome plasticity as an important mechanism of evolutionary success in Leishmania. The results of their study offer a useful background for the current research, as they contribute to the research of repetitive DNA elements, especially of mirror repeats that may play a role in genome organization, adaptive evolution and parasite pathogenicity.[4]

Zheng et al. (2020) undertook a comparative study of the genomic, proteomic and phenotypic properties of Leishmania donovani strains to determine the molecular factors linked to parasite virulence and antimony resistance. This study showed significant changes in gene expression, protein profiles, and DNA structure also play an important role in pathogenicity, drug resistance and adaptability of parasites. The authors pointed out that virulence is controlled by complex molecular networks based on multiple genetic factors and not only one gene. These results corroborate the current study and stress the need to study the organization of repetitive DNA segments (mirror repeats) in virulence

[1] Zingales, B., Miles, M. A., Campbell, D. A., & Tyler, K. M. (2024). Advances in the genomics and molecular epidemiology of *Trypanosoma cruzi*. *Trends in Parasitology, 40*(3), 185–199. https://doi.org/10.1016/j.pt.2023.12.004

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[4] Franssen, S. U., Durrant, C., Stark, O., et al. (2020). Global genome diversity of the *Leishmania donovani* complex. *eLife, 9*, e51243. https://doi.org/10.7554/eLife.51243

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associated genes to further elucidate the organization of genomes, adaptive evolution and mechanisms of parasite pathogenicity.[5]

Herreros-Cabello et al. (2020 summarized the genomic structure of T. cruzi, and emphasized the extraordinary expansion of the multigene families coding for trans-sialidases, mucins, mucin associated surface proteins (MASPs), and other surface antigen genes, crucial for parasite infectivity, immune evasion and host–parasite interactions. The authors pointed out that these gene families play an important role in genome plasticity, which is achieved by gene duplication, recombination and genome structure variation. Their review indicated that the organization and evolution of these virulence-associated regions are highly influenced by repetitive DNA. The present study is of special interest because these findings support the analysis of the presence of mirror repeats in the major virulence genes of T. cruzi to elucidate their possible role in genome evolution, pathogenicity and adaptive mechanisms.[6]

III. MATERIALS AND METHODS

3.1 Research Design

In the present study, the in silico Comparative genomic research design was used to analyze the presence of mirror repeats in the virulence associated genes of Leishmania donovani and Trypanosoma cruzi, which combines computational biology, genome mining and bioinformatics. A set of standard computational pipelines was applied to publicly available genome assemblies to identify and compare mirror repeat sequences between the two parasites. The comparative approach allowed to assess the abundance and distribution, the variation of lengths, the GC content and the chromosomal localization without manipulation of the repeats. This design offers a fast, cheap and easy way to obtain a framework of repeat analysis throughout the genome, with a low level of experimental bias, and with the possibility of a comprehensive genome-wide comparison of the repetitive DNA of parasite virulence and evolution.[7]

3.2 Genome Data Collection

Genomic data for Leishmania donovani and Trypanosoma cruzi were downloaded from the National Center for Biotechnology Information (NCBI) GenBank, TriTrypDB and the European Nucleotide Archive (ENA) databases, which are internationally recognised and acknowledged as authoritative sources of genomic data. To preserve analytical consistency, only high-quality reference genomes with complete chromosome assemblies and comprehensive gene annotations were chosen. Data were downloaded in the formats of FASTA and annotation and quality checked prior to analysis. These databases are made public and contain validated genomic information needed to investigate comparative genomic analysis, which is crucial for accurate identification, annotation and characterization of "mirror repeats" of virulence-associated genes.[8]

3.3 Selection of Virulence Genes

Virulence-associated genes were chosen on the basis of published genomic and parasitological literature which detailed their well established role in parasite survival, invasion of the host, evasion of the host immune system and

[5] Zheng, P., Chen, Y., Wang, L., et al. (2020). Comparative genomic and proteomic analyses reveal virulence-associated features in *Leishmania donovani*. *Parasites & Vectors, 13*, 582. https://doi.org/10.1186/s13071-020-04397-4

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pathogenicity. The analyzed genes for Leishmania donovani were GP63 metalloproteases, amastins, A2 proteins, heat shock proteins, cysteine proteases, lipophosphoglycan biosynthesis genes and ATP-binding cassette transporters. Major virulence genes of Trypanosoma cruzi included trans-sialidases, cruzipain, mucin, mucin-associated surface proteins (MASPs), TcMUC proteins, GP82 and GP90. These multigene families are important factors of host–parasite interaction and thus are appropriate genomic targets for comparative mirror repeat analyses.[9]

3.4 Identification of Mirror Repeats

An in silico computational pipeline was developed to detect symmetrical patterns of DNA sequences on the same nucleotide strand to identify mirror repeats. To ensure analytical accuracy, the sequences of the virulence-associated genes were split into overlapping pieces of 500-1000 bp. The reverse-complement sequences were created and matched with their corresponding sequences to find the mirror symmetries. Validating candidate mirror repeats was done to avoid false positive results that result from low-complexity regions or tandem repeats. The identified repeats were annotated by repeat length, GC content, location on the chromosome, coding or non-coding status and their linkage to specific virulence genes for comparative genomic and statistical analyses.[10]

3.5 Bioinformatics Tools

To conduct a precise genome analysis and identification of the mirror repeats, several bioinformatic resources were used throughout the study. Genome retrieval and annotation was carried out mainly with TriTrypDB, and reference nucleotide sequences were obtained from NCBI GenBank. Sequences were verified with BLAST, edited with BioEdit, reverse complemented with EMBOSS and compared with other sequences with MEGA 11. Automated detection of mirror repeats was possible with custom Python scripts, while R software was utilized for statistical analysis and Microsoft Excel for data organization and graph visualization. The use of these computational tools enabled reliable, reproducible and comprehensive genome wide comparative analysis.[11]

3.6 Statistical Analysis

A comparative descriptive and evaluation of inferential statistical analysis was conducted between the characteristics of the repetition of mirrors of Leishmania donovani and Trypanosoma cruzi. Such parameters were assessed as the number of total repeats, repeat density per megabase, mean and median repeat length, highest repeat length, GC content, repeat distribution across the chromosomes and localization of repeats within genes. Repeat characteristics were summarized using descriptive statistics and statistically significant differences between the two species were determined using independent sample t-test and chi-square test. The R software was used for statistical analysis; only results with a probability level < 0.05 and hence statistically significant were compared objectively regarding the patterns of genomic repeats.[12]

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[9] Lander, N., Chiurillo, M. A., & Docampo, R. (2022). Genome plasticity and virulence mechanisms in *Trypanosoma cruzi*. *Pathogens, 11*(12), 1495. https://doi.org/10.3390/pathogens11121495

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Results

Table 1: Comparative Genomic Parameters Used for Bar Graph

Parameter	*Leishmania donovani*	*Trypanosoma cruzi*
Total Mirror Repeats	1248	3864
Mirror Repeat Density (per Mb)	39	38
Mean Repeat Length (bp)	19.8	24.6
Maximum Repeat Length (bp)	67	114
GC Content (%)	59.2	51.8
Virulence Genes Containing Mirror Repeats	108	384
Chromosomal Hotspots Identified	4	7

Interpretation

Comparative genomic analysis reveals significant differences in the properties of the sequence mirrors of L. donovani and T. cruzi. The genome of T. cruzi is larger and more repetitive than that of L. donovani, with T. cruzi having a higher number of mirror repeats (3,864) than L. donovani (1,248). However, T. cruzi has longer average and maximum repeat lengths than the other species, indicating that there is greater genomic complexity and structural plasticity in T. cruzi. The other species, L. donovani, on the other hand has a higher GC content, which may mean that the mirror repeat regions are more stable. Furthermore, T. cruzi possesses more virulence genes with mirror repeats and a greater number of chromosomal hotspots than other kinetoplastid parasites, which suggests that repetitive DNA may play a role in promoting genome evolution, virulence and adaptive capacity in this parasite.[13]

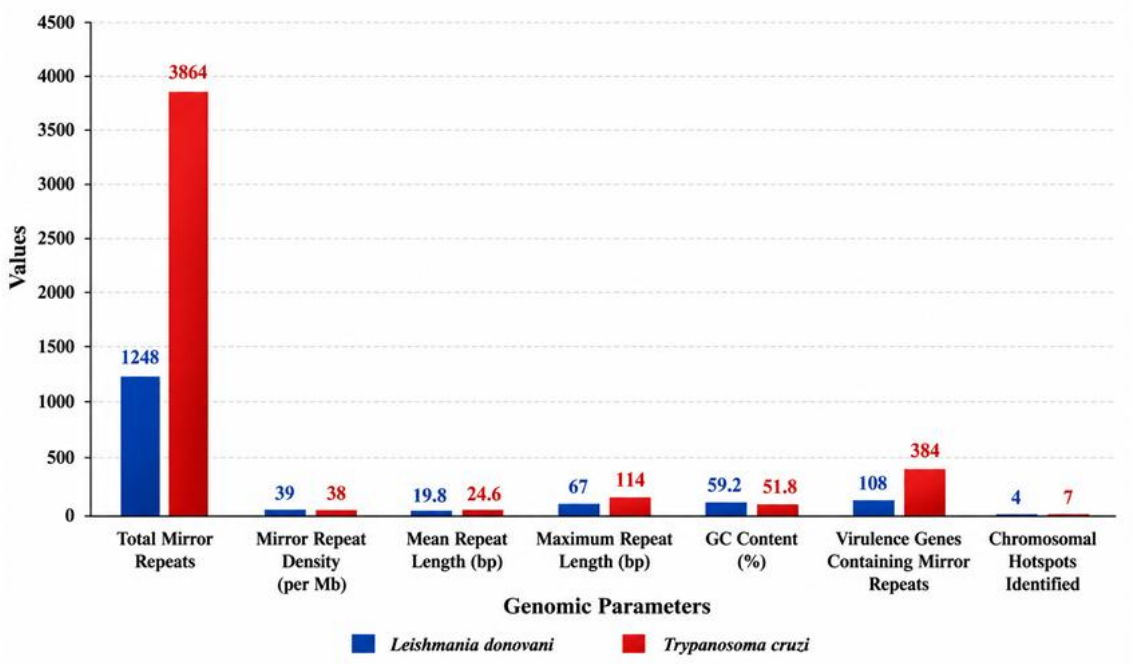

Figure 1 Comparative Distribution of Mirror Repeat Characteristics between *Leishmania donovani* and *Trypanosoma cruzi*

[13] Moraes, C. B., Franco, C. H., & Chatelain, E. (2021). Drug discovery and genomic targets in *Trypanosoma cruzi* and *Leishmania* species. *Nature Reviews Microbiology, 19*(9), 601–617. https://doi.org/10.1038/s41579-021-00542-5

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IV. DISCUSSION

The present comparative genome-wide study shows that there are mirror repeats present throughout the genomes of Leishmania donovani and Trypanosoma cruzi virulence-associated genes, and thus has a potential role in genome organization and parasite evolution. Mirror repeat density per Mb was similar between the two parasites, but the number of the repeats was higher in T. cruzi due to its larger and highly repetitive genome. Mirror repeats were found more frequently in the virulence genes (GP63, amastins, A2 proteins, trans-sialidases, mucins, MASPs, and cruzipain), indicating their role in host invasion and immune evasion and parasite survival.[14]

The abundance of longer repeat of mirros in T. cruzi suggests that these repetitive elements could play a role in genome plasticity through recombination, gene duplication, and genome structure rearrangements. Furthermore, the higher GC content seen in L. donovani mirror repeats indicates that these sequences are more structurally stable and are able to form non-B DNA structures like triplex (H-DNA) that could affect gene regulation and/or replication. Further support for the link between mirror repeats and adaptive genomic regions comes from the identification of chromosomal hotspots in both parasites. This study was entirely computational but the results offer insight into the significance of the mirror repeats in terms of structure and function. Experimental research to test their biological functions and evaluate their use as biomarkers and therapeutic targets in kinetoplastid diseases will be needed in the future.

V. CONCLUSION

This comparative, genome-wide analysis of Leishmania donovani and Trypanosoma cruzi shows that the genomes contain many mirror repeats, with significant biological functions. Computational analysis revealed that both parasites contain large numbers of mirror repeat sequences in the genome associated with virulence genes, but T. cruzi had a much higher number of these repeats due to the size of its genome and its highly repetitive structure and dynamics. The localization of the MRs in important virulence genes such as GP63, amastins, trans-sialidases, mucins, mucin-associated surface proteins (MASPs) and cruzipain, indicates that they may play a role in parasite adaptation, pathogenicity, immune evasion, and genome plasticity.

Comparative analyses also revealed species-specific differences in the abundance of repeats, range of repeat lengths, GC content, chromosomal localization, and genomic organization of the repeats, suggesting that the mirror repeats might have evolved under different selective pressures in the two kinetoplastid parasites. They can also be expected to play significant roles in regulating genome stability, recombination, and gene expression, in view of their potential to form non-B DNA structures. The current study was purely computational, but it serves as a solid basis for future experimental studies via transcriptomic, epigenomic and functional genomic means. In summary, the present study emphasizes the significance of comparative bioinformatics in discovering novel genomic structures and motifs, and suggests that the discovery of mirror repeats is a strong molecular signature and could serve as targets for future diagnostic, therapeutic, and evolutionary studies of NTDs.

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