

## Genetic Diversity, Plasticity, and Gene Manipulation Strategies in *Leishmania*: Implications for Treatment and Vaccine Research

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### ABSTRACT

Leishmaniasis is a neglected tropical disease (NTD) that remains a significant global health challenge. While a small number of drugs are clinically effective, their use is constrained by toxicity, high cost, and emerging drug resistance. Furthermore, no licensed human vaccine is currently available. *Leishmania* exhibits extensive genomic plasticity, primarily through aneuploidy, gene copy number variations (CNVs), and subtelomeric amplifications, which confer remarkable flexibility and rapid adaptation to adverse environments, such as those encountered within the host or during drug treatment. Moreover, the extensive genetic diversity and plasticity of *Leishmania* enable it to rapidly adjust gene expression in response to environmental changes, facilitating adaptation to various stressors, including host immune responses and drug exposure. This adaptability significantly complicates effective treatment. In addition to vertical gene transfer, genetic exchange through hybridization and karyotypic instability are well-documented mechanisms that enhance the parasite's capacity to adapt to environmental stress. The role of horizontal gene transfer remains under investigation. This mini-review highlights the unique genomic features of *Leishmania*, particularly its genetic flexibility and unconventional mechanisms of gene expression regulation, such as aneuploidy and CNVs. It also examines major genetic manipulation tools used to investigate parasite biology, while discussing their inherent limitations. Understanding gene function is essential yet remains challenging due to the parasite's atypical genome organization and regulatory mechanisms. A deeper comprehension of *Leishmania* genetics is therefore crucial for the rational design of effective drugs and vaccines.

### INTRODUCTION

Leishmaniasis is a vector-borne disease caused by *Leishmania* parasites, which are transmitted to vertebrate hosts, including humans, through the bite of infected sand flies. It manifests in several clinical forms, including cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL). These clinical forms occur in both the Old World and New World, varying according to the infecting *Leishmania* species and the sand fly vector involved. The host immune response strongly influences disease severity. Leishmaniasis is endemic in Africa, Asia, including the Middle East, Latin America, and parts of Southern Europe [1]. Despite its global distribution and burden, leishmaniasis is classified as a neglected tropical disease (NTD) due to limited research and funding attention, the absence of effective vaccines, and current treatments

characterized by significant toxicity, high cost, and emerging drug resistance [2].

*Leishmania* is a unicellular eukaryotic parasite belonging to the order Kinetoplastida and the family Trypanosomatidae. The genus *Leishmania* includes approximately 53 identified species, of which more than 20 are known to infect humans [3]. These human-pathogenic species infect various mammals, including humans, dogs, and rodents, causing different clinical forms of leishmaniasis. Most *Leishmania* species are zoonotic, maintaining animal reservoirs alongside human infections, whereas some species are anthroponotic, relying solely on humans as reservoirs, such as *L. tropica* and *L. donovani* [4]. Recently, a novel *Leishmania* species (*L. ellisi*), which causes CL, was identified in the United States [5, 6], suggesting that the known species diversity within this genus continues to expand. Interestingly,

unlike human-pathogenic species of *Leishmania*, the reptile-associated *L. tarentolae* is non-pathogenic to humans [3]. This trait, along with several other advantages, renders *L. tarentolae* a valuable model organism for biotechnological and biomedical applications [7-9].

This mini-review provides an overview of the genetic diversity and genomic plasticity in *Leishmania* species, elucidating the underlying causes of this diversity, its association with drug resistance, and the gene manipulation tools employed to investigate parasite biology. It also discusses the challenges these factors pose for the development of effective therapies and vaccines against leishmaniasis.

### Comparative biology: *Leishmania*, eukaryotes, and prokaryotes

As a unicellular eukaryote, the *Leishmania* parasite exhibits a unique combination of prokaryotic and eukaryotic characteristics, as summarized in Table 1. Like prokaryotes and other unicellular eukaryotes, *Leishmania* is unicellular, in contrast to most multicellular higher eukaryotes. Additionally, like eukaryotes, *Leishmania* genes are organized into diploid chromosomes. In higher

eukaryotes, each protein-coding gene has its own promoter and is transcribed as a monocistronic unit. In contrast, *Leishmania* protein-coding genes are organized into polycistronic transcription units in a head-to-tail fashion, similar to prokaryotic operons, where multiple genes are transcribed together from a single start site. In *Leishmania*, genes are transcribed into precursor mRNA (pre-mRNA) by RNA polymerase II from upstream promoter regions that drive transcription of the entire polycistron, rather than from individual, gene-specific promoters. As a result, gene expression is regulated post-transcriptionally by sequences in the untranslated regions (UTRs) adjacent to each gene. Furthermore, *Leishmania* genes generally lack introns and undergo trans-splicing, where a conserved 39-nucleotide spliced leader (SL) sequence is added to the 5' end of each pre-mRNA transcript; this process is coupled with polyadenylation at the 3' end [10], resulting in mature monocistronic mRNAs [11]. Another notable feature of protein expression in *Leishmania* is its capacity for post-translational modifications (PTMs), including glycosylation patterns that resemble those observed in human cells [3]. Notably, this capability has made the non-pathogenic *L. tarentolae* an attractive expression platform in medical biotechnology [12, 13].

**Table 1.** Comparative biological features of *Leishmania*, higher eukaryotes, and prokaryotes.

Features	<i>Leishmania</i>	Higher eukaryotes	Prokaryotes
Cell type	Unicellular	Multicellular	Unicellular
Nuclear membrane	Yes	Yes	No
Chr <sup>a</sup>	Yes	Yes	No, nucleoid region
Ploidy/Genome structure	Diploid/aneuploid/polyploid	Diploid/polyploid	Typically a single circular chromosome
Transcription unit	Polycistronic	Mostly monocistronic	Polycistronic
Introns	Rare/absent	Yes	Absent
Splicing type	Trans	Cis and trans	None
RNAP II promoter <sup>b</sup>	No gene-specific promoters	Yes	N/A (uses distinct RNA polymerase)
PTMs <sup>c</sup>	Yes	Yes	Yes, but limited

<sup>a</sup> Chr: Chromosome.

<sup>b</sup> RNAP II promoter: RNA polymerase II-specific promoter.

<sup>c</sup> PTMs: Post-translational modifications.

### *Leishmania* genome: organization, aneuploidy, and copy number variations

*Leishmania* parasites exhibit distinctive genomic organization, characterized by chromosomal variability and genetic plasticity. The number of chromosomes (Chr) varies among species: Old World species typically have 36, whereas New World species have 34 or 35, typically resulting from chromosomal fusions (Table 2). The *Leishmania* genome contains over 8,000 genes, with species-specific differences often arising from the presence or absence of particular gene sets [14]. The subgenus *Sauroleishmania*, which includes *L. tarentolae* and *L. adleri*, is restricted to the Old World species and primarily infects lizards. These species are either non-pathogenic or capable of causing only transient infections in humans [3]. Although *Sauroleishmania* species share

approximately 90% genomic identity with human-pathogenic species, they lack key virulence-associated genes, such as the A2 gene [3, 15].

Furthermore, the *Leishmania* genome is typically diploid, with genes present in two or multiple copies. However, under certain conditions, gene copy numbers can change, and chromosomes may shift between euploid and aneuploid states, reflecting the parasite's genomic plasticity. Moreover, unlike most eukaryotes, *Leishmania* largely lacks conventional gene-specific transcriptional regulation. Instead, it relies on alternative mechanisms, including genomic rearrangements, copy number variations (CNVs), and aneuploidy, which enable dosage modulation. Mosaic aneuploidy, in which chromosome numbers vary among individual cells within a population, is particularly notable [16]. Changes in chromosome or

gene copy number correlate directly with transcript levels in a dosage-dependent manner, enabling the parasite to fine-tune gene expression according to environmental conditions. This mode of regulation is facilitated by abundant genomic repeats, including repetitive sequences (direct and inverted repeats) and short interspersed degenerate retroposons (SIDERs). These elements play a key role in promoting copy number changes and driving genomic plasticity. In most organisms, particularly humans, aneuploidy is typically deleterious, often associated with developmental disorders and diseases, including cancer and Down syndrome. In contrast, *Leishmania* has uniquely adapted to exploit aneuploidy as a survival strategy. Although predominantly diploid, the parasite can tolerate a wide range of chromosome copy numbers (somes), from monosomy (one copy) to hexasomy (six copies), both constitutively and in response to stress conditions [17]. Remarkably, this genomic plasticity is not only tolerated but also advantageous. It enables the parasite to withstand adverse environments,

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including host immune pressure and drug treatment. Moreover, mosaic aneuploidy and genetic heterogeneity are distinctive features of *Leishmania* populations. Consequently, individual cells within a population can possess different chromosomal copy numbers, resulting in significant intra-population diversity. Indeed, extensive genome diversity has been reported in most *Leishmania* species. Such diversity generates multiple phenotypes within a single population, providing a reservoir of adaptability that enhances long-term survival. Genetic hybridization between different *Leishmania* species and the potential for sexual reproduction in the parasite's life cycle are important mechanisms of genetic exchange. These processes contribute to diversity and adaptation [18]. However, despite the benefits of genomic plasticity, cells also utilize a range of post-transcriptional and translational regulatory mechanisms, including non-coding RNAs such as small nucleolar RNAs (snoRNAs), to attenuate the potentially deleterious consequences of gene copy number alterations [18].

**Table 2.** Genetic characteristics of selected *Leishmania* species from the Old and New Worlds.

Species	Chr <sup>a</sup>	Genome size	Gene count	Chr fusion <sup>b</sup>	Clinical manifestation/pathogenicity
<b>Old World</b>					
<i>L. major</i>	36	32.8 Mb	8,272	–	CL <sup>c</sup>
<i>L. infantum</i>	36	32.1 Mb	8,154	–	VL <sup>d</sup>
<i>L. tropica</i>	36	32.9 Mb	8,133	–	CL, VL
<i>L. donovani</i>	36	32.4 Mb	8,032	–	VL
<i>L. aethiopica</i>	36	31.6 Mb	~8,100	–	CL
<i>L. tarentolae</i> (TAR)	36	31.6 Mb	~8,200	–	Non-pathogenic to humans
<i>L. tarentolae</i> (LEM)	36	31.6 Mb	~8,200	–	Transient/nonspecific infection
<i>L. adleri</i>	38	30.4 Mb	7,849	–	Non-pathogenic to humans
<b>New World</b>					
<i>L. mexicana</i>	34	31.4 Mb	8,250	8+29, 30+36	CL, VL, MCL <sup>e</sup>
<i>L. amazonensis</i>	34	32.2 Mb	8,317	–	CL
<i>L. braziliensis</i>	35	32.3 Mb	8,395	20+34	CL, VL, MCL
<i>L. guyanensis</i>	35	30.5 Mb	8,273	–	CL
<i>L. panamensis</i>	35	30.7 Mb	7,933	–	CL

<sup>a</sup> Chr: Chromosomes; <sup>b</sup> Chr fusion: Chromosomal fusion events, <sup>c</sup> CL: Cutaneous leishmaniasis, <sup>d</sup> VL: Visceral leishmaniasis and <sup>e</sup> MCL: Mucocutaneous leishmaniasis. –: No chromosomal fusion events reported.

#### Treatment complications: genetic diversity, plasticity, and drug resistance

The treatment of leishmaniasis faces significant challenges due to inconsistent drug efficacy and the emergence of resistance. The parasite's extensive genetic variation and genomic instability exacerbate this problem. The genomic plasticity of *Leishmania* enables rapid adaptation to environmental pressures, including drug exposure, which promotes the emergence of drug resistance. Additionally, phenomena such as genome reorganization and the development of clonal variants allow *Leishmania* to undergo genomic alterations, enhancing its adaptability to changing environmental conditions. These dynamic genomic changes and flexible gene expression mechanisms present major obstacles to leishmaniasis treatment, particularly in the context of emerging drug resistance [19]. Moreover, genomic studies have revealed a strong correlation between CNVs and drug resistance in *Leishmania*, suggesting that CNVs may serve as biomarkers for assessing drug susceptibility

[20]. A more detailed understanding of the genetic diversity and genomic plasticity of *Leishmania* is crucial for identifying key proteins and metabolic pathways linked to drug resistance, which may serve as novel therapeutic targets and enable the development of innovative treatments [20].

#### Gene function studies: gene manipulation approaches

As mentioned earlier, *Leishmania* harbors more than 8,000 genes, the functions of the majority of which remain unknown. To determine the role of these genes, one effective approach is reverse genetics, in which one or more alleles at loci in diploid or polyploid genomes are disrupted or removed. Understanding gene function in *Leishmania* through gene overexpression or knockout approaches is critical for deciphering its biology and identifying potential therapeutic targets. Researchers have developed several gene manipulation tools to assess how genetic changes affect the parasite's survival, virulence, and treatment responses, as described below.

A) Gene knockout (KO) or gene disruption by homologous recombination (HR): This approach involves the complete deletion or inactivation of a gene to study its loss-of-function effects. It is widely used to identify genes essential for *Leishmania's* survival and/or infectivity within the host. Gene replacement using antibiotic resistance genes through HR was the first technique developed for this purpose in *Leishmania*, introduced in 1995 [21]. Between 2007 and 2015, this method was extensively used to identify genes essential for the parasite's survival [22]. Although HR is still widely used and has proven effective, its limited efficiency and speed make it less suitable for high-throughput gene studies.

B) CRISPR/Cas9 genome editing system: Since 2015, this system has revolutionized genetic manipulation by enabling precise edits to the *Leishmania* genome and has become a widely used approach for investigating gene function. This tool enables targeted gene knockouts, insertions, or modifications, providing greater accuracy and versatility in functional studies. CRISPR/Cas9 approaches include both traditional gene editing, which relies on double-strand breaks [23, 24], and more recently developed CRISPR base editing techniques, which enable single-base edits without inducing double-strand breaks [25, 26]. To date, approximately 250 genes have been functionally characterized, primarily in pathogenic species such as *L. major* and *L. donovani* [22]. Notably, researchers have used the CRISPR/Cas9 system to disrupt genes that could not be successfully mutated using HR [27].

C) RNA Interference (RNAi): This technique is a powerful tool for transiently silencing gene expression by introducing small interfering RNAs (siRNAs). It allows researchers to study the effects of reduced gene expression. This technique helps elucidate the functional roles of genes in the parasite's life cycle and stress responses. However, it cannot be applied to most *Leishmania* species, including *L. major* and *L. donovani*, which lack functional RNAi machinery. Notably, *L. braziliensis* is an exception, as it retains a functional RNAi pathway [28].

In addition to knockout approaches, overexpression studies are also employed. In these studies, researchers introduce additional copies of specific genes using episomal vectors, HR, or CRISPR/Cas9 systems to assess how increased gene expression levels affect *Leishmania's* phenotype. Overexpression studies are useful for identifying genes involved in drug resistance, virulence, and stress responses, as well as for understanding the regulation of specific pathways.

These approaches allow researchers to compare transgenic and wild-type strains. Following gene editing, the next challenge is determining whether the gene is essential or dispensable for the parasite's survival and infectivity in either the promastigote or amastigote stage. Studying the amastigote stage is particularly important, given its role in mammalian infection. Nevertheless,

studying gene functions in *Leishmania* presents challenges. Due to the parasite's digenetic life cycle—alternating between the promastigote (in sand flies) and amastigote (in mammalian hosts) stages—investigating the role of individual genes is complex and time-consuming. Despite these challenges, gene deletion remains pivotal for identifying essential genes, which are prime candidates for drug target identification and vaccine development.

Deleting and validating essential genes pose significant challenges. Creating null mutants by removing both alleles is often lethal, and even heterozygous knockout strains may exhibit reduced fitness or viability. A potential solution is to introduce an extra copy of the gene—either episomal or ectopic—into the parasite to enable gene deletion. However, this approach carries a risk of inducing aneuploidy, which requires careful genomic analysis to verify genomic integrity. Furthermore, to validate the essential role of a gene in infectivity, it is crucial to study the amastigote stage of the mutant strain using *in vitro* or *in vivo* models.

## CONCLUSION

Investigating leishmaniasis is exceptionally complex, and progress in developing control measures has lagged behind that for other major infectious diseases such as malaria. Several factors contribute to this, including: 1) the complex biology of the parasite, its digenetic life cycle, and its ability to adapt to diverse environments; 2) the intricate interactions between the parasite, the sand fly vector, and the mammalian host, which vary depending on parasite species and host genetics; 3) the presence of multiple *Leishmania* species and the diverse clinical forms of leishmaniasis; 4) the lack of suitable animal models that fully replicate human infection; and 5) the classification of leishmaniasis as a neglected tropical disease. Despite these challenges, researchers continue to work toward developing effective drugs and vaccines against leishmaniasis. To make meaningful progress, a comprehensive understanding of *Leishmania's* biology and the roles of its genes is essential. While evaluating each gene individually remains necessary, the process is both challenging and time-consuming. Following genetic manipulations, it is crucial to examine the tripartite interactions between the parasite, the sand fly vector, and the mammalian host. Accelerating these studies is therefore critical. Moreover, strategic use of modern technologies can facilitate this research. Integrating bioinformatics tools, artificial intelligence, and techniques such as fluorescence *in situ* hybridization (FISH) can help prioritize gene targets before experimental studies. Furthermore, multi-omics analyses (*e.g.*, transcriptomics, proteomics, metabolomics) following genetic manipulation can significantly enhance the efficiency and depth of drug and vaccine discovery pipelines.

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## CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest associated with this manuscript.

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## AI DISCLOSURE

No artificial intelligence tools were used in the preparation of this manuscript.

## DATA AVAILABILITY

This manuscript is a mini-review article. All data and information discussed are presented within the manuscript and derived from previously published studies, which are cited accordingly. No new datasets were generated or analyzed during this study.

## AUTHORS' CONTRIBUTIONS

TT: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

## ETHICS STATEMENT

Ethical approval was not required for this study as it is a review article that did not involve human subjects, animal experiments, or primary data collection.

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