

Lotmaria passim as an experimental model of trypanosomatid adaptation to the insect gut

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Abstract

Lotmaria passim is a monoxenous trypanosomatid that persistently colonizes the hindgut of the western honey bee, *Apis mellifera*. Recent chromosome-level genome resources, reverse genetics, conditional protein depletion, omics approaches and experimental infection have shifted research on this parasite from detection surveys towards mechanistic analysis. Here, we synthesize evidence that genome plasticity, flagellar remodeling, Bardet–Biedl syndrome protein complex (BBSome)-dependent membrane organization, ergosterol biosynthesis and extracellular secretion collectively support parasite growth and hindgut colonization. Functional studies reveal that phenotypes observed in culture do not necessarily predict host fitness: loss of duplicated flagellar calcium-binding proteins impairs morphology and motility without substantially reducing infection, whereas disruption of BBS2, sterol C5-desaturase or the secreted aspartyl protease LpAsp compromises honey bee colonization. These findings identify membrane homeostasis, protein trafficking and extracellular functions, rather than motility alone, as important determinants of infection. We further propose *L. passim* as a comparative model for dissecting conserved insect-stage mechanisms shared with other monoxenous trypanosomatids and the insect stages of *Leishmania* and *Trypanosoma cruzi*. Resolving the signals and molecular machinery controlling haptomonad differentiation, attachment and extracellular community formation will clarify how trypanosomatids adapt to persistent extracellular growth in insect digestive tracts.

1. Introduction

Trypanosomatids are flagellated protists that parasitize a broad range of invertebrates, vertebrates and plants. Although research has traditionally concentrated on dioxenous species responsible for human and animal diseases, most trypanosomatids are monoxenous parasites of invertebrates (Lukeš et al. 2018; Maslov et al. 2019). These insect-associated species provide important comparative systems for identifying the ancestral cellular features of trypanosomatids and understanding how particular lineages subsequently acquired the capacity to alternate between insect and vertebrate hosts. Nevertheless, the molecular biology of most monoxenous trypanosomatids remains poorly characterized because relatively few species can be continuously cultured, genetically manipulated and examined using reproducible experimental infection models.

Lotmaria passim is a monoxenous trypanosomatid that colonizes the digestive tract of the western honey bee, *Apis mellifera*. The parasite was formally distinguished from *Crithidia mellificae* in 2015 on the basis of morphological, ultrastructural, molecular and phylogenetic evidence (Schwarz et al. 2015). Species-specific molecular analyses further demonstrated that *L. passim* is considerably more prevalent than *C. mellificae* in honey bee populations and is widely distributed across geographically separated apicultural regions (Ravoet et al. 2015). The parasite is therefore now regarded as the predominant trypanosomatid associated with *A. mellifera*.

The consequences of *L. passim* infection for honey bee health remain incompletely resolved. Experimental studies have reported reduced survival, altered nutritional physiology and host gene

expression, and increased responsiveness to sucrose, but outcomes differ among parasite isolates, culture histories and experimental conditions (Gómez-Moracho et al. 2020; Buendía-Abad et al. 2021; Liu et al. 2020; MacInnis, Luong, and Pernal 2023). Infection also occurs within a complex hindgut ecosystem containing a specialized bacterial community, dietary components and other microorganisms, making it difficult to distinguish direct parasite-mediated effects from context-dependent interactions. The proposed life cycle of *L. passim* is summarized in Fig. 1.

Genomic resources and the development of reverse genetics have transformed *L. passim* from a parasite studied mainly through detection surveys into an experimentally tractable system for molecular and biochemical parasitology. Its biology raises fundamental questions concerning how a monoxenous trypanosomatid coordinates genome plasticity, membrane composition, flagellar remodeling, protein trafficking, secretion and metabolic adaptation to establish persistent extracellular infection in an insect digestive tract. The honey bee hindgut is a spatially structured, largely hypoxic and acidic environment in which the resident microbiota transforms pollen-derived substrates and produces organic acids and other metabolites (Liu et al. 2020; Zheng et al. 2017; Kešnerová et al. 2017; Callegari et al. 2021), providing a distinctive setting in which to investigate trypanosomatid adaptation.

Recent reviews have comprehensively considered the epidemiology, pathology, transmission, host responses and broader biology of bee-associated trypanosomatids (Tiritelli, Cilia, and Gómez-Moracho 2025; García Olmedo et al. 2025). The present review therefore adopts a more focused parasite-centered perspective. We consider the value of *L. passim* as a comparative model for distinguishing conserved trypanosomatid processes from lineage-specific adaptations associated with monoxenous parasitism.

2. Genome architecture and genome plasticity

Genomic resources for *L. passim* have progressed from an early fragmented draft assembly of strain SF (Runckel, DeRisi, and Flenniken 2014) to a chromosome-level reference genome for the BRL type strain and additional draft genomes from geographically distinct isolates (Ruiz et al. 2025; Markowitz et al. 2025; Nearman et al. 2025). These resources have established a framework for gene annotation, comparative genomics and reverse-genetic studies. However, differences in reported genome size and gene number among isolates should be interpreted cautiously because the assemblies were generated using different sequencing and annotation methods. Uniform analysis of additional low-passage isolates will be required to distinguish genuine biological variation from technical differences.

The nuclear genome retains the characteristic organization of trypanosomatids. Protein-coding genes are densely arranged in long directional clusters that are transcribed as polycistronic precursor RNAs. Individual mRNA abundance is therefore regulated predominantly through trans-splicing, polyadenylation, RNA stability, translation and protein turnover rather than conventional gene-specific transcriptional initiation. Extensive conservation of gene order with *Leptomonas*, *Crithidia* and *Leishmania* indicates that the overall genome architecture is evolutionarily stable, despite lineage-specific chromosome rearrangements.

The mitochondrial genome is organized as kinetoplast DNA containing maxicircle and minicircle molecules. The chromosome-level assembly includes one maxicircle and 30 minicircle sequences (Markowitz et al. 2025). In other trypanosomatids, maxicircles encode mitochondrial proteins and ribosomal RNAs, whereas minicircles encode guide RNAs that direct uridine insertion/deletion editing of maxicircle-derived transcripts (Pollard et al. 1990). The minicircle repertoire and mitochondrial RNA-editing patterns of *L. passim* have not yet been characterized experimentally.

A prominent feature of the *L. passim* genome is variation in chromosome copy number, or aneusomy. Most chromosomes in the BRL strain are predominantly disomic, but several show increased or reduced copy number. Comparisons of samples collected after prolonged laboratory cultivation indicate that chromosome dosage may change over time, possibly through duplication of individual homologues or shifts in the abundance of karyotypically distinct subpopulations. Such flexibility is common among trypanosomatids and may provide a rapid mechanism for altering the dosage of many genes simultaneously.

The biological significance of these changes remains uncertain. Somy variation could contribute to adaptation to culture conditions, environmental stress or the honey bee hindgut, but direct evidence for adaptive selection is currently lacking.

Chromosomes 5 and 6 represent a notable example of structural genome evolution, apparently arising through duplication of an ancestral chromosome followed by sequence and gene-content divergence. Genes on these chromosomes are disproportionately induced during honey bee infection and include predicted surface proteins, peptidases, transporters, metabolic enzymes and multiple GP63-like metalloproteases, suggesting possible roles in extracellular growth and host colonization. However, this association remains correlative, and the contribution of these duplicated chromosomes to infection must be tested through functional analysis of selected genes and comparisons among parasite isolates.

Overall, *L. passim* combines a conserved trypanosomatid genome organization with substantial structural flexibility. Chromosome duplication, somy variation and paralogue divergence may generate phenotypic diversity despite the limited role of gene-specific transcriptional regulation. The chromosome-level reference genome now permits these genomic features to be integrated with transcriptomic, biochemical and reverse-genetic data. The major challenge is to determine whether genome plasticity merely reflects laboratory instability or contributes directly to parasite growth, differentiation and persistent host colonization.

3. Experimental tools for functional analysis

The development of culture, genetic manipulation and experimental infection methods has transformed *L. passim* into a tractable model for molecular parasitology. The parasite can be propagated axenically, cloned, genetically modified and reintroduced into its natural honey bee host, allowing genes identified through genomic or omics analyses to be evaluated at the molecular, cellular and infection levels.

However, the available methods remain less efficient and scalable than those established for major medical trypanosomatids. The experimental workflow is summarized in Fig. 2 and Table 1.

Table 1
Genomic and experimental resources available for functional analysis of *Lotmaria passim*.

Resource or method	Principal application	Demonstrated use
Chromosome-level genomes	Gene annotation and comparative genomics	Identification of somy variation and duplicated chromosomes
Axenic cultivation	Growth and biochemical studies	Morphology, motility, stress and drug assays
Episomal expression	Protein localization and complementation	GFP, tdTomato and epitope-tagged proteins
CRISPR/Cas9 disruption	Analysis of nonessential genes	FCaBPs, BBS2, LpAsp, LpCht and SC5Ds
Endogenous tagging	Native protein localization and regulation	LpBBS1 tagging
Auxin-inducible degron	Conditional analysis of essential proteins	LpBBS1 depletion
Transcriptomics	Infection responses and mutant phenotypes	Dual RNA-seq and BBS2-mutant analysis
Proteomics and metabolite analysis	Extracellular proteins and metabolism	Exoproteome and sterol profiling
Oral honey bee infection	Host-colonization phenotype	Functional testing of mutants

3.1 Axenic cultivation and cellular phenotyping

L. passim promastigotes can be maintained continuously in modified FP-FB medium (Salathé et al. 2012). Parasites have been cultured at approximately 21–30°C, depending on the experimental objective. Axenic culture permits quantitative analysis of growth, cell and flagellar morphology, motility, aggregation, stress tolerance and drug susceptibility. Parasites can be cloned by limiting dilution or growth on semisolid medium. Culture conditions strongly influence parasite phenotypes. For example, defects associated with altered sterol composition or BBSome function become more pronounced at lower temperatures (Yuan and Kadowaki 2025, 2026). Moreover, nutrient-rich aerobic culture differs substantially from the honey bee hindgut environment. The absence of a phenotype under a single culture condition therefore does not establish that a gene is dispensable during infection.

3.2 Transfection, protein expression and genome editing

Stable transfection of *L. passim* was established by electroporation of promastigotes with plasmids derived from trypanosomatid expression systems. Regulatory sequences originally developed for *Trypanosoma cruzi* and *Leishmania* are functional in *L. passim*, permitting expression of fluorescent

proteins, Cas9, selectable markers and tagged parasite proteins. Because transient transfection efficiency is low, stable transformants are generally recovered by antibiotic selection.

Episomal expression of GFP, tdTomato and epitope-tagged proteins has been used to examine protein localization and complement mutant phenotypes. Episomal complementation is particularly useful for confirming gene-specific phenotypes, although variation in plasmid copy number and expression level can complicate quantitative interpretation. Endogenous tagging is therefore preferable when native protein abundance or localization is important. Proximity-dependent biotinylation has also been demonstrated using the engineered biotin ligase ultraID. This approach detected proximity between the N-terminal sorting sequence of LpFCaBP1 and LpBBS1, showing that proximity labelling can be applied to protein-interaction studies in *L. passim* (Yuan and Kadowaki 2024). However, fusion tags can alter protein localization or function and must be validated carefully.

Targeted gene disruption is performed using a Cas9-expressing parasite line, a gene-specific guide RNA and donor DNA containing a selectable marker flanked by sequences homologous to the target locus. Cas9-induced cleavage promotes homologous replacement of endogenous genes and has been used to disrupt genes encoding a miltefosine transporter, tyrosine aminotransferase, duplicated flagellar calcium-binding proteins, secreted hydrolases, BBS2 and sterol C5-desaturase. At least two independent mutant clones and a complemented strain are desirable because clonal variation or unintended genomic changes may influence phenotype. Failure to recover a homozygous mutant may indicate essentiality, but inefficient guide activity, inaccurate genome annotation or additional gene copies must first be excluded. The present system usually replaces the target locus with a drug-resistance cassette. Although robust, this limits the number of sequential modifications and may affect neighbouring genes within polycistronic transcription units. Marker recycling, scarless editing and transient Cas9–guide delivery have not yet been established. Endogenous tagging by homology-directed repair has nevertheless been demonstrated, including introduction of epitope and degron sequences at the *LpBBS1* locus (Yuan and Kadowaki 2025).

3.3 Conditional protein depletion

Constitutive deletion cannot be used to investigate genes required for parasite viability. This limitation was partly overcome by adapting the auxin-inducible degron system to *L. passim*. In this system, the target protein is tagged with minilAA7, and parasites express the plant F-box protein AFB2. Addition of auxin promotes ubiquitination and proteasomal degradation of the tagged protein. The system was first validated using a minilAA7–GFP reporter and was subsequently applied to LpBBS1 after homozygous deletion mutants could not be recovered. Auxin-dependent depletion of LpBBS1 reduced parasite growth, supporting an essential or near-essential role for this BBSome component (Yuan and Kadowaki 2025). The auxin-inducible degron currently represents the principal conditional loss-of-function method available for *L. passim*. Its effectiveness may vary according to protein abundance, stability and subcellular localization, and tagging may itself partially alter protein function. Application during honey bee infection is also challenging because auxin must reach parasites in the hindgut without affecting the

host or microbiota. Additional conditional systems will therefore be required to analyse essential proteins directly during colonization.

3.4 Transcriptomic, proteomic and biochemical approaches

Transcriptomics has been used to examine both parasite adaptation during infection and the consequences of targeted gene disruption. Dual RNA sequencing of infected honey bees revealed temporal changes in parasite genes associated with translation, energy metabolism, mitochondrial activity, oxidative stress and surface functions. Because parasite RNA may represent only a small fraction of total hindgut RNA, particularly during early infection, adequate sequencing depth and biological replication are essential (Liu et al. 2020). RNA sequencing of genetically modified parasites can reveal secondary and compensatory responses not apparent from growth or morphology alone. For example, deletion of *LpBBS2* altered genes associated with metabolism, microtubules, mitochondrial function, DNA repair and membrane-protein localization. Interpretation of these datasets nevertheless depends on accurate and consistent genome annotation (Yuan and Kadowaki 2025).

Proteomic analysis of conditioned medium identified a reference *L. passim* exoproteome and provided candidate extracellular proteins for functional testing. Genetic analysis subsequently showed that a secreted aspartyl protease contributes to parasite growth and honey bee infection. Biochemical approaches have linked specific metabolic pathways with cellular and infection phenotypes. Gas chromatography–mass spectrometry demonstrated that ergosterol is the major sterol of wild-type parasites and identified accumulation of ergosta-7,22-dienol after deletion of sterol C5-desaturase. Combining genetic disruption, metabolite measurement, drug susceptibility and infection assays directly connected sterol composition with parasite fitness.

3.5 Experimental honey bee infection

A reproducible oral infection model provides the critical connection between parasite molecular biology and colonization of the natural host. Logarithmically growing parasites are fed individually to newly emerged workers at 10^5 cells per bee. Infected bees are maintained in laboratory cages, and parasite abundance is typically measured after 14 days by quantitative PCR targeting the *L. passim* ITS2 region of ribosomal RNA gene and normalized to a honey bee genomic reference.

Quantitative PCR is sensitive and suitable for analyzing large numbers of bees, but it measures parasite DNA rather than viable cells and does not identify the affected stage of infection (Vejnovic et al. 2018). Reduced parasite abundance may result from impaired survival after ingestion, failure to reach the hindgut, defective attachment, reduced differentiation or slower proliferation. Fluorescent reporters, microscopy and tissue-specific quantification should therefore be combined with qPCR when the mechanism of an infection phenotype is unclear (Liu, Lei, and Kadowaki 2019; Buendía-Abad et al. 2022). Infection outcomes can vary among individual bees and are influenced by host genetic background, nutritional and rearing conditions, and microbiota composition (Schwarz, Moran, and Evans 2016).

Parasite growth phase and culture-passage history can also affect infectivity or pathogenicity (Gómez-Moracho et al. 2020; Buendía-Abad et al. 2021). Experiments should therefore include independent cages or colony sources, sufficient biological replication, multiple mutant clones and complemented controls.

4. Molecular and biochemical mechanisms underlying growth and hindgut colonization

Successful colonization of the honey bee hindgut requires *L. passim* to coordinate developmental differentiation, membrane organization, extracellular secretion and adaptation to the gut environment. Current evidence indicates that these processes act together rather than through a single dominant virulence factor. Functionally characterized genes are summarized in Table 2.

Table 2
Functionally characterized genes and molecular pathways in *Lotmaria passim*.

Gene or pathway	Manipulation	Principal cellular phenotype	Honey bee infection phenotype	References
LpFCaBP1 and LpFCaBP2	Double deletion and complementation	Reduced growth, altered morphology and motility	Little or no substantial reduction	21
LpBBS1	Conditional depletion	Reduced growth	Not fully resolved during conditional infection	19
LpBBS2	Gene deletion	Reduced size and motility; temperature-sensitive growth; altered membrane organization	Reduced colonization	19
LpSC8I	Attempted deletion	Homozygous mutant not recovered	Not determined	20
LpSC5D1 and LpSC5D2	Double deletion and complementation	Loss of ergosterol, altered morphology and temperature sensitivity	Reduced colonization	20
LpAsp	Gene deletion	Altered growth or aggregation	Reduced colonization	27
LpCht	Gene deletion	No major phenotype under tested conditions	No clear reduction	27

4.1 Promastigote–haptomonad differentiation

L. passim occurs predominantly as elongated, motile promastigotes during axenic growth. In the honey bee hindgut, parasites develop into haptomonad forms in which the flagellum is shortened and

remodelled into a specialized attachment structure. Haptomonad-like communities can also develop in culture following extracellular-matrix production and surface attachment. These structures share important features with microbial biofilms, including stable adhesion, multicellular organization and increased stress resistance. However, the signals initiating differentiation, the composition of the attachment structure and the molecular markers distinguishing developmental stages remain unknown.

The transition between promastigotes and haptomonads is therefore likely to be central to the balance between persistence and transmission. Attached haptomonads are adapted for stable hindgut colonization, whereas free promastigotes are better suited to dispersal between hosts (Buendía-Abad et al. 2022; Carreira de Paula et al. 2024).

4.2 Flagellar and membrane organization

The flagellum of *L. passim* contributes to morphogenesis, membrane organization and attachment in addition to motility. The duplicated flagellar calcium-binding proteins LpFCaBP1 and LpFCaBP2 illustrate how related proteins can acquire different subcellular distributions. LpFCaBP1 is enriched in the flagellum, whereas LpFCaBP2 is distributed across the entire parasite membrane. Despite these differences, the two proteins retain partly overlapping functions in growth, cell shape, flagellar morphology and motility. Loss of both FCaBPs impairs several cellular traits without substantially reducing honey bee infection. Thus, moderate reductions in motility and flagellar length are not necessarily sufficient to prevent colonization. This suggests that other flagellar functions, particularly protein trafficking, membrane organization and developmental remodeling, may be more important in the host. The BBSome appears to contribute to these functions. LpBBS1 is required for normal parasite growth, whereas loss of LpBBS2 causes reduced cell and flagellar size, impaired motility, temperature-sensitive growth and inefficient honey bee colonization. BBS2 deficiency also weakens the association of acylated proteins with sterol-rich membrane domains and partially alters the flagellar enrichment of an LpFCaBP1-derived reporter. These findings suggest that the *L. passim* BBSome helps maintain membrane-domain organization and the distribution of flagellar proteins. Its importance for infection probably extends beyond motility and may involve the trafficking of proteins required for environmental tolerance, differentiation or attachment (Yuan and Kadowaki 2024, 2025).

4.3 Sterol metabolism and membrane homeostasis

Ergosterol is the predominant sterol of *L. passim* and contributes to membrane structure and function. Sterol C8-isomerase appears to be essential for viability, whereas the two duplicated sterol C5-desaturases, LpSC5D1 and LpSC5D2, retain overlapping enzymatic activity. Parasites lacking both SC5D genes cannot produce ergosterol and instead accumulate ergosta-7,22-dienol. Replacement of ergosterol with this precursor permits nearly normal growth under favourable conditions but causes abnormal morphology, temperature-sensitive growth and resistance to amphotericin B. More importantly, ergosterol-deficient parasites colonize honey bees inefficiently. Ergosterol is therefore not absolutely required for cell division in culture but becomes critical under environmental stress and within the host. Sterol composition may affect membrane permeability, transport, flagellar function and developmental

differentiation. It may also be functionally linked to the BBSome because both ergosterol deficiency and BBS2 loss disrupt membrane-associated processes and reduce infection. Sterol-rich membrane domains could provide the physical environment required for BBSome-dependent localization or retention of proteins involved in hindgut colonization (Yuan and Kadowaki 2026).

4.4 Secreted factors and extracellular material

L. passim releases a diverse range of extracellular proteins, including proteases, antioxidant proteins and metabolic enzymes. Some are conserved in the extracellular proteomes of other Leishmaniinae, suggesting that they perform ancestral functions in extracellular survival, nutrient acquisition or surface remodeling. The secreted aspartyl protease LpAsp contributes to parasite growth and honey bee colonization. Its physiological substrate is unknown, but altered aggregation in LpAsp-deficient parasites suggests that it may regulate cell–cell or cell–surface interactions. In contrast, a predicted secreted chitinase is dispensable under standard experimental conditions, indicating that the presence of a secreted enzyme does not necessarily imply an essential role in infection. Extracellular polymeric material associated with promastigotes and haptomonad communities may stabilize attachment, protect against environmental stress and retain enzymes or nutrients near the parasite surface. Vesicle-like structures have also been observed within this material, although their composition and biological functions remain unresolved (Yuan, Sun, and Kadowaki 2024; Carreira de Paula et al. 2024).

4.5 Adaptation to the honey bee hindgut

The honey bee hindgut presents fluctuating conditions of pH, oxygen availability, osmolarity and nutrient supply and contains a dense bacterial microbiota (Zheng et al. 2017; Kešnerová et al. 2017; Callegari et al. 2021). During infection, *L. passim* alters pathways associated with energy metabolism, mitochondrial activity, oxidative-stress responses and surface functions, consistent with progressive adaptation to this environment (Liu et al. 2020). The mechanisms described above provide complementary forms of protection. Haptomonad differentiation and extracellular-matrix production resist displacement by gut flow. Ergosterol supports membrane function under stress, while BBSome-dependent trafficking maintains membrane and flagellar organization. Secreted proteins may promote nutrient acquisition and modify interactions with host tissues or resident bacteria. The microbiota may either restrict the parasite through nutrient competition, acidification and antimicrobial metabolites or support it by providing fermentation products and other nutrients. However, direct molecular interactions between *L. passim* and individual gut bacterial species remain largely unexplored. An integrated model is presented in Fig. 3.

5. *L. passim* as a comparative model for trypanosomatid biology

The significance of *L. passim* extends beyond honey bee health. As a monoxenous member of the Leishmaniinae, it provides a tractable system for investigating molecular adaptations that allow trypanosomatids to grow extracellularly, differentiate, attach and persist within insect digestive tracts. Its

genome retains extensive synteny with *Leptomonas*, *Crithidia* and *Leishmania*, indicating that its specialized biology has developed within a broadly conserved trypanosomatid genomic framework.

A major experimental advantage of *L. passim* is that genetically modified parasites can be characterized in culture and subsequently tested in their natural host. This makes it possible to distinguish general cellular functions from traits specifically required for infection. For example, impaired morphology or motility does not necessarily reduce honey bee colonization, whereas disruption of membrane trafficking or sterol metabolism can produce relatively modest culture phenotypes but substantial infection defects. Thus, *L. passim* provides a direct means of evaluating the host relevance of conserved trypanosomatid pathways.

Comparisons with other bee-associated trypanosomatids may clarify the molecular basis of host and tissue specialization. *C. mellificae* occupies a similar hindgut niche in honey bees, whereas *Crithidia bombi* colonizes the bumblebee hindgut. These parasites share extracellular growth, flagellar attachment and fecal–oral transmission but differ in host range, morphology and prevalence. Comparative genomics and functional analysis could determine whether attachment, metabolism and stress adaptation rely on conserved mechanisms or parasite-specific innovations.

L. passim also provides a useful comparison with the insect stages of dixenous trypanosomatids. During *Leishmania* development in sand flies, promastigotes differentiate into haptomonads that attach to the stomodeal valve through a shortened and extensively remodelled flagellum containing a specialized adhesion plaque (Yanase et al. 2023; Yanase et al. 2024). Similarly, *Trypanosoma cruzi* epimastigotes attach through the terminal region of the flagellum to the rectal cuticle of triatomine insects, and this attachment is closely associated with differentiation into metacyclic trypomastigotes (Kleffmann, Schmidt, and Schaub 1998; Figueiredo, Rosa, and Soares 2000). These similarities are consistent with the hypothesis that flagellar remodeling and attachment are ancestral insect-stage traits that were retained and modified during the evolution of dixenous parasitism. The relatively simple life cycle of *L. passim*, which lacks a vertebrate stage, may therefore facilitate analysis of the fundamental mechanisms underlying these processes.

The principal limitations of *L. passim* as a comparative model are the small number of experimentally studied strains, possible genomic changes during prolonged cultivation and the lack of methods for isolating defined developmental stages from infected bees. Nevertheless, it combines conserved trypanosomatid cell biology with a natural insect infection model and direct genetic testing of parasite determinants. Comparative studies using *L. passim* can therefore help distinguish ancestral mechanisms of insect-gut colonization from lineage-specific adaptations associated with particular hosts and with the emergence of vertebrate-infective trypanosomatids.

6. Conclusions and future research priorities

L. passim hindgut colonization depends on coordinated developmental differentiation, membrane and flagellar organization, sterol metabolism and extracellular functions, and infection phenotypes cannot

always be predicted from growth or motility in axenic culture.

Several central questions remain unresolved. The signals controlling haptomonad differentiation, the molecular composition of the attachment structure and extracellular matrix, the relevant BBSome cargoes and the functions of most secreted proteins are unknown. The nutritional requirements of the parasite and its direct interactions with the honey bee gut microbiota also require investigation. Further progress will require improvement and standardization of experimental resources. Current genome editing is slow, depends on drug-resistance markers and is not yet suitable for large-scale functional screening. Marker recycling, scarless editing, additional conditional systems and standardized tagging constructs would accelerate genetic analysis. Methods that resolve individual infection stages should be prioritized. Stable reporter strains, live imaging of infected hindguts, isolation of attached haptomonads and direct recovery of viable parasites from bees would permit stage-specific genomic, proteomic and metabolic analyses.

The major opportunity is now to connect parasite genotype, molecular function, biochemical state and developmental form within the natural host. With continued methodological development, *L. passim* can provide a valuable comparative model for understanding how trypanosomatids adapt to insect digestive tracts and how ancestral insect-stage mechanisms contributed to the evolution of more complex trypanosomatid life cycles.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Consent to participate

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Author Contribution

T.K. conceived the review, evaluated the literature and drafted the manuscript. Q.L., J.L., X.Y. contributed to literature analysis and critically revised the manuscript. All authors approved the final manuscript.

Data availability

Data sharing is not applicable to this article because no datasets were generated.

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Figures

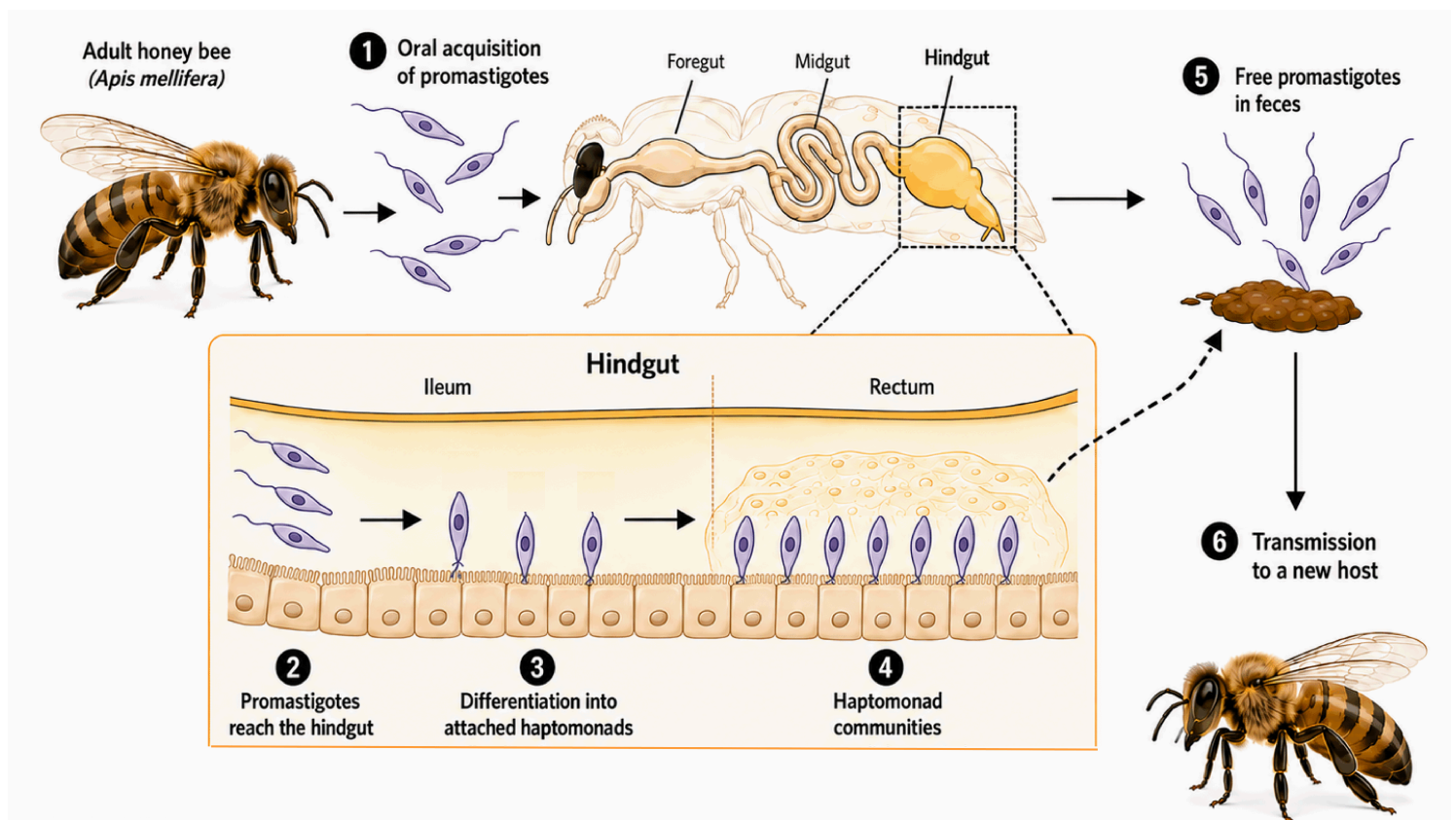


Figure 1

Life cycle and hindgut colonization of *Lotmaria passim*.

Adult honey bees acquire motile promastigotes orally from contaminated material. Following passage through the digestive tract, promastigotes reach the hindgut, where the anterior flagellum contacts the luminal surface and is progressively shortened and remodeled to form the attachment structure of the haptomonad stage. Attached haptomonads develop into extracellular-matrix-associated, biofilm-like communities; for simplicity, these communities are illustrated in the rectum. Free promastigotes released in feces are proposed to mediate transmission to new hosts. Solid arrows indicate experimentally supported transitions, whereas dashed arrows indicate inferred or incompletely resolved transitions.

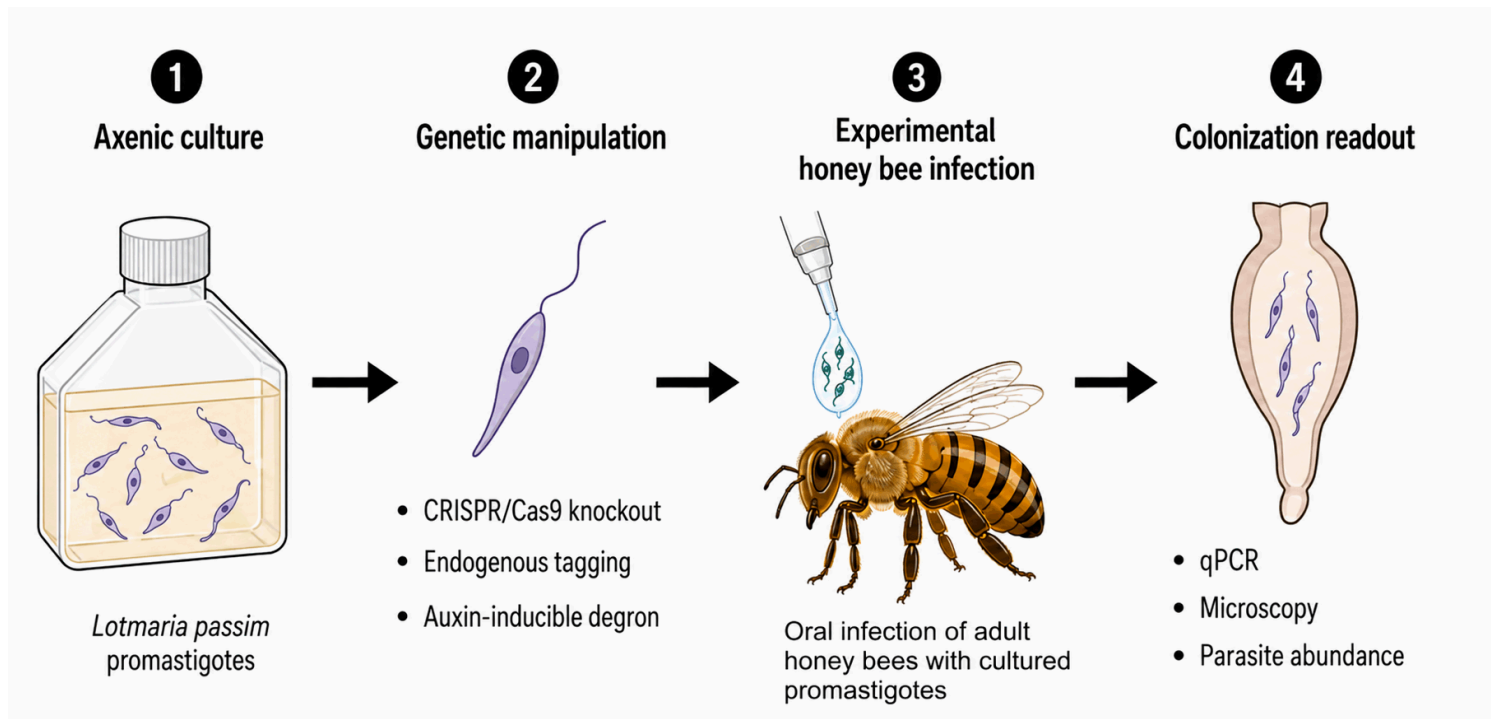


Figure 2

Experimental platform for functional analysis of *Lotmaria passim*.

The experimental workflow combines axenic cultivation of promastigotes with genetic manipulation, including CRISPR/Cas9-mediated gene disruption, endogenous tagging and auxin-inducible protein depletion. Genetically modified parasites are characterized by cellular, molecular and biochemical analyses and subsequently administered orally to adult honey bees. Hindgut colonization is evaluated using quantitative PCR, microscopy and parasite-abundance measurements.

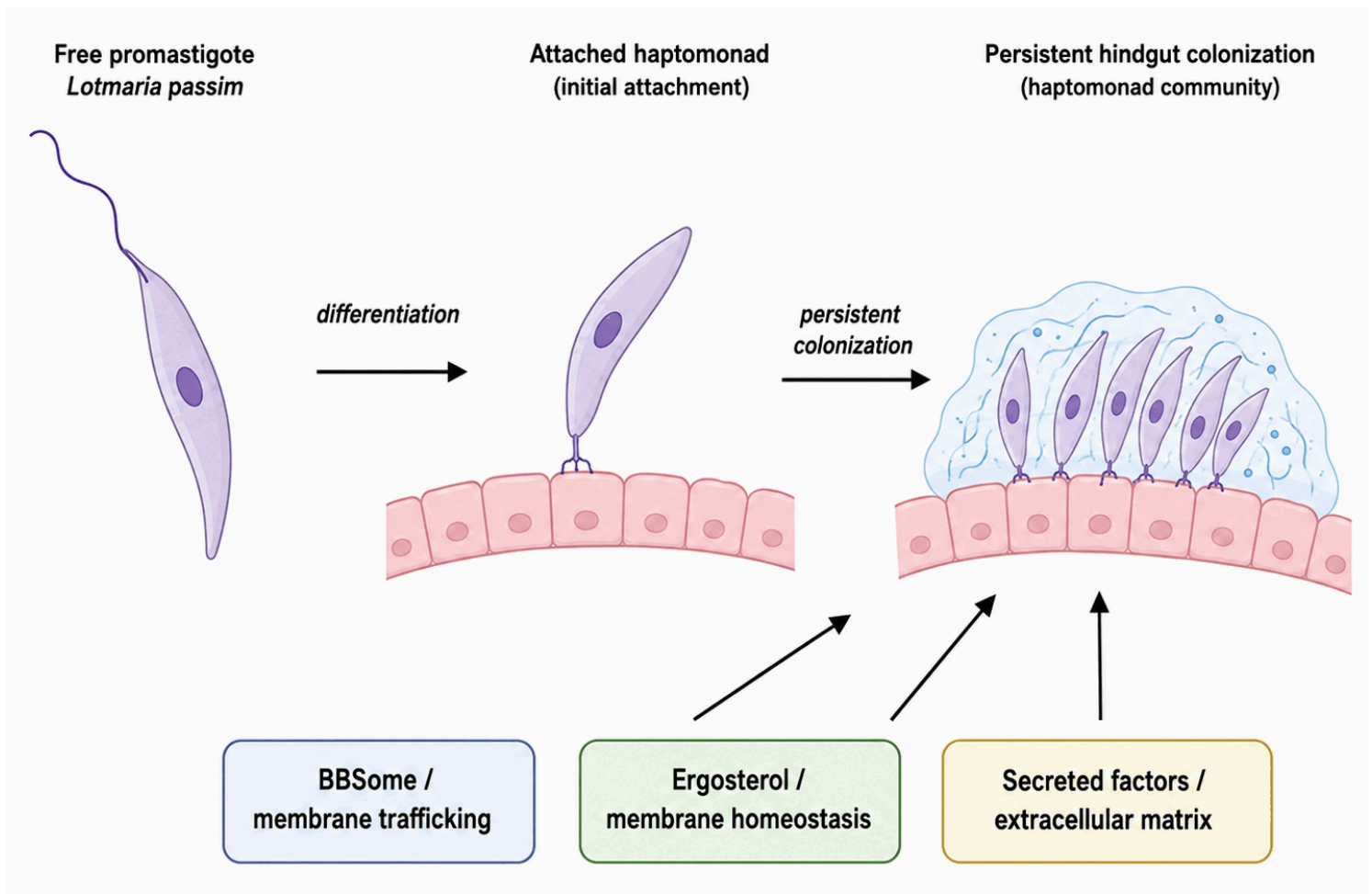


Figure 3

Integrated model of *Lotmaria passim* hindgut colonization.

Free promastigotes differentiate into haptomonads after the anterior flagellum attaches to the hindgut surface and undergoes extensive shortening and structural remodeling. Attached haptomonads subsequently establish persistent extracellular-matrix-associated communities. BBSome-dependent membrane trafficking and flagellar organization, ergosterol-dependent membrane homeostasis, and secreted factors such as extracellular proteins and matrix components are proposed to act together to support attachment, environmental tolerance and persistent colonization. The model summarizes experimentally supported findings and mechanistic relationships that remain to be tested directly.