
REGULATION OF THE *Acanthamoeba castellanii* GENOME UPON INFECTION BY *Legionella pneumophila*

A PREPRINT

September 7, 2021

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Keywords *Acanthamoeba castellanii*, *Legionella pneumophila*, genome organization, assembly, infection

ABSTRACT

The unicellular amoeba *Acanthamoeba castellanii* is ubiquitous in aquatic environments where it predares on bacteria. However, this protozoa also hosts bacterial endosymbionts and intracellular bacteria that are parasites of amoeba. These include human pathogens, such as *Chlamydia* or *Legionella* spp. Here we report the complete genome sequence for *A. castellanii* strains Neff and C3, two extensively studied strains. Using a hybrid approach combining long reads, Hi-C and shotgun sequencing we generated a near chromosome-level assembly of both strains with 90% of the genome comprised in 29 scaffolds for the Neff strain and 31 for the C3 strain. This gave us the unique possibility for comparative genomics of *A. castellanii* and revealed high sequence divergence. Core metabolism genes are largely conserved whereas important differences in signal transduction were identified. Furthermore, we characterize the spatial organization of *A. castellanii* and show that it is reorganized during infection by *Legionella pneumophila*, a protozoan parasite and feared human pathogen. We find infection dependent chromatin loops to be enriched in signal transduction and phosphorylation processes. Enrichment of structural changes at metabolism and organelle assembly, microtubule and cytoskeleton organization suggest that chromosomal folding is associated with many changes in host cells observed during infection.

Keywords Genomics · Assembly · Comparative genomics · Hi-C · Host-parasite

Introduction

The first amoeba were isolated in 1913 [1], and the genus *Acanthamoeba* was established in 1931 by Volkonsky [2]. It comprises different species of free living, aerobic, unicellular protozoa, present throughout the world in soil and

nearly all aquatic environments [3]. The life cycle of *Acanthamoeba* includes a dormant cyst with minimal metabolic activities under harsh condition and a motile trophozoite that can feed on small organisms and that reproduce by binary fission in optimal conditions [4]. In rare cases, *Acanthamoeba* species may be a pathogen for humans causing serious infections of the central nervous system, lungs, sinuses and skin, mostly in immunocompromised humans, but also a vision-threatening disease, *Acanthamoeba* keratitis [5]. The species *Acanthamoeba castellanii* was first isolated in 1930 by Castellanii as a contaminant of yeast.

In their natural environment, they are characterized by the ability to change their shape through pseudopode formation and are considered professional phagocytes as they are feeding on bacteria, but may also phagocytize yeasts or algae. However, some bacteria are resistant to degradation and live as endosymbionts in these protozoa, and others even use amoeba as a replication niche. Thus *Acanthamoeba* are also reservoirs of microorganisms and viruses, including human pathogens, which have adapted to survive inside these cells and resist digestion, persist or even replicate as intracellular parasites. At least 15 different bacterial species, two archaea and several eukaryotes and viruses have been shown to interact with *Acanthamoeba* in the environment and may even co-exist at the same time in the same host cell [6].

Although it was observed early on that bacteria could resist digestion of free-living amoebae [7], it was not until the discovery that *Legionella pneumophila* replicated in amoebae that the bacteria-amoeba relationship began to be studied in depth [8]. *L. pneumophila* is the agent responsible for the legionnaire's disease, a severe pneumonia that can be fatal if not treated promptly. In addition, many species of amoeba have the ability to form highly resistant cysts in hostile environments, providing shelter for their intracellular parasites [9]. Indeed, it is thought that *L. pneumophila* may survive water disinfection treatments and contaminate water distribution systems thanks to cysts protection [10, 11, 12]. From these contaminated water sources, *L. pneumophila* can reach the human lungs via aerosols contaminated with the bacteria and replicate within the alveolar macrophages that are, like amoeba, phagocytic cells.

L. pneumophila has the ability to escape the lysosomal degradation pathway of both *A. castellanii* and human alveolar macrophages through the formation of a protective vacuole (the Legionella-containing vacuole or LCV) where it multiplies to high numbers. Once the host cell is exploited and nutrients become limited, *L. pneumophila* exits the host to start an infection in a new cell [13].

To establish the LCV and replicate, *L. pneumophila* secretes over 300 effector proteins via a type four secretion system (T4SS) called Dot/Icm [14] into the host cytoplasm to manipulate the host pathways and redirect nutrients to the LCV [15, 16]. In the early stages of infection, many of these proteins target the host secretory pathway, including several small GTPases, to recruit the endoplasmic reticulum [17]. During the intracellular cycle, a wider range of processes, including membrane trafficking, cytoskeleton dynamics or signal transduction pathways, are targeted by these effectors [18, 19]. *L. pneumophila* also directly alters the genome of its host, by modifying epigenetic marks of the host genome in human macrophages and in *A. castellanii*. It secretes an effector named RomA that encodes histone methyltransferase activity in the host cell, where it is targeted to the nucleus. RomA trimethylates K14 of histone H3 [20] genome wide, leading to transcriptional changes that modulate the host response in favor of the survival of the bacteria [20]. Concomitantly, *L. pneumophila* infection leads to genome-wide changes in gene expression [21]. In many eukaryotes, gene regulation is intertwined with the tridimensional organization of chromosomes, and whether chromatin loops, self-interacting domains, and/or active/inactive compartments play roles in cellular development, response to environmental changes, or processes such as DNA repair, are actively being studied [22, 23]. Therefore, the infection of *A. castellanii* by *L. pneumophila* provides an amenable model to investigate how an intracellular bacterial infection may affect the regulation of chromosome folding, and its consequences, in a eukaryotic host.

The investigation of the genome organization and regulatory states of *A. castellanii* in response to infection requires a highly contiguous genome assembly. The current genome reference of *A. castellanii* (NEFF-v1, [24] is based on the Neff strain, isolated from soil in California in 1957 [25], that is widely used in different laboratories studying *A. castellanii*. This assembly is fragmented into 384 scaffolds, which makes chromosome-level analyses difficult, if not impossible. In addition, some general features of the *A. castellanii* genome, such as the number of chromosomes or the ploidy, remain undetermined. In addition, many teams investigating bacteria-amoeba interactions use the "C3" strain (ATCC 50739), isolated from a drinking water reservoir in Europe in 1994 and identified as mouse pathogen [26]. This, despite scarce genomic information and little information available regarding its phylogenetic distance to the Neff strain. Notably, the extent of genome conservation between these two *A. castellanii* strains that have been cultivated for several decades and were isolated from different ecological niches is unknown. The lack of genomic resources hampers the investigation of the factors determining the susceptibility of the pathogen to different *A. castellanii* strains, as well as the application of genome-wide omics approaches.

In this work, we aimed at characterising the response to infection of *A. castellanii* C3 strain by *L. pneumophila* through the prism of the three-dimensional organisation of the amoeba genome. This analysis required the generation of a high quality reference genome sequence of the C3 strain, which we then compared to a new assembly of the Neff reference strain also presented in this paper. Illumina shotgun, Nanopore long read, and Hi-C were used to generate

strain	step	length	scaffolds	N50	L50	N90	L90
C3	01-flye	46527779	368	406024	28	82982	122
C3	02-hypo	46287494	368	402530	28	82062	122
C3	03-instagraal	46287494	253	1424952	13	895233	29
C3	04-pilon	46273248	225	1424952	13	895233	29
C3	05-manual	46256395	227	1400265	14	895233	31
C3	06-rcorr	46255455	227	1400134	14	895207	31
Neff	01-flye	44412593	237	1159072	15	141936	51
Neff	02-hypo	44503078	237	1161784	15	142434	51
Neff	03-instagraal	44503078	179	1460606	13	965190	28
Neff	04-pilon	44504078	179	1460763	13	965290	28
Neff	05-manual	43957095	178	1332098	14	911389	29
Neff	06-rcorr	43956874	178	1332098	14	911382	29

Table 1: Assembly statistics for *A. castellanii* at each pipeline step. For both strains, general assembly metrics are shown alongside the name of the tool used in that step and its position within the pipeline. Manual denotes manually curated changes.

near chromosome-level assemblies of both strains. The new Neff and C3 assemblies have a (gap-excluded) sequence divergence of 6.7%. We find that core metabolism genes are largely conserved between the two strains and differences in gene content are mostly associated with signal transduction. Using the C3 assembly, RNA-seq and Hi-C, we were able to analyze the genome folding and expression changes of *A. castellanii* in response to the infection by *L. pneumophila*. We found infection-dependent chromatin loops to be enriched in signal transduction and phosphorylation.

Results

The genome assemblies for *A. castellanii* Neff and C3 are highly contiguous and complete

We used a combination of ONT long reads, Hi-C and shotgun sequencing to reassemble the genome at chromosome level, with 90% of the genome comprised in 28 scaffolds. This is in contrast to the previously estimated number of chromosomes of around 20 using pulsed-field gel electrophoresis [27]. For both the Neff and C3 strains, we first generated a raw *de-novo* assembly using Oxford Nanopore long reads, of 44.4 and 46.5 Mb in size, respectively (Table 1). To account for the error prone nature of long reads, we combined them with paired-end shotgun Illumina sequences to polish the first draft assembly using HyPo [28]. The polished assembly was then scaffolded with long range Hi-C contacts using our probabilistic program instaGRAAL, which exploits a Markov Chain Monte Carlo algorithm to swap DNA segments until the likeliest scaffold is achieved [29]. Following the post-scaffolding polishing step of the program (see [29]), the final assemblies of both strains displayed a better contiguity, completion and mapping statistics than the previous version of the assembly, with the cumulative length of the scaffolds quickly reaching a plateau (Fig. 1a). The assemblies of both strains are also slightly longer, with a smaller number of contigs than the previous Neff assembly (NEFF-v1) (Fig. 1b). The BUSCO-completeness of the assembly is also improved, with 90.6% (Neff) and 91.8% (C3) complete eukaryotic universal single copy orthologs, compared to 77.6% for NEFF-v1. Hi-C contact maps consist in a convenient readout to explore large misassemblies in genome sequences [30]. While this allowed us to manually address major unambiguous misassemblies, a number of visible misassemblies remain in complex regions such as repeated sequences near telomeres or rDNAs. In C3, there are also a few (at least 5) interchromosomal misassemblies which appear to be heterozygous and cannot be resolved without a phased genome. We also found shotgun coverage to be highly heterogeneous between scaffolds, which is suggestive of aneuploidies (Fig. S1).

Comparison of Neff and C3 strains identifies differences in ...

As we had assembled the genomes of two different *A. castellanii* strains for the first time, we had the unique opportunity to compare them. We used Orthofinder to compare the gene content of the C3 and Neff strains. We found xxx (mannose-binding lectin + gene content details) xx.

Spatial organisation of the *A. castellanii* genome

To our knowledge, no Hi-C contact maps have been generated from species of the Amoebozoa clade. Therefore, the Hi-C reads used to generate the chromosome scale scaffolding of two *A. castellanii* strains also offer the opportunity to expose the average genome folding in a species of this clade. Hi-C reads were realigned along the new assemblies

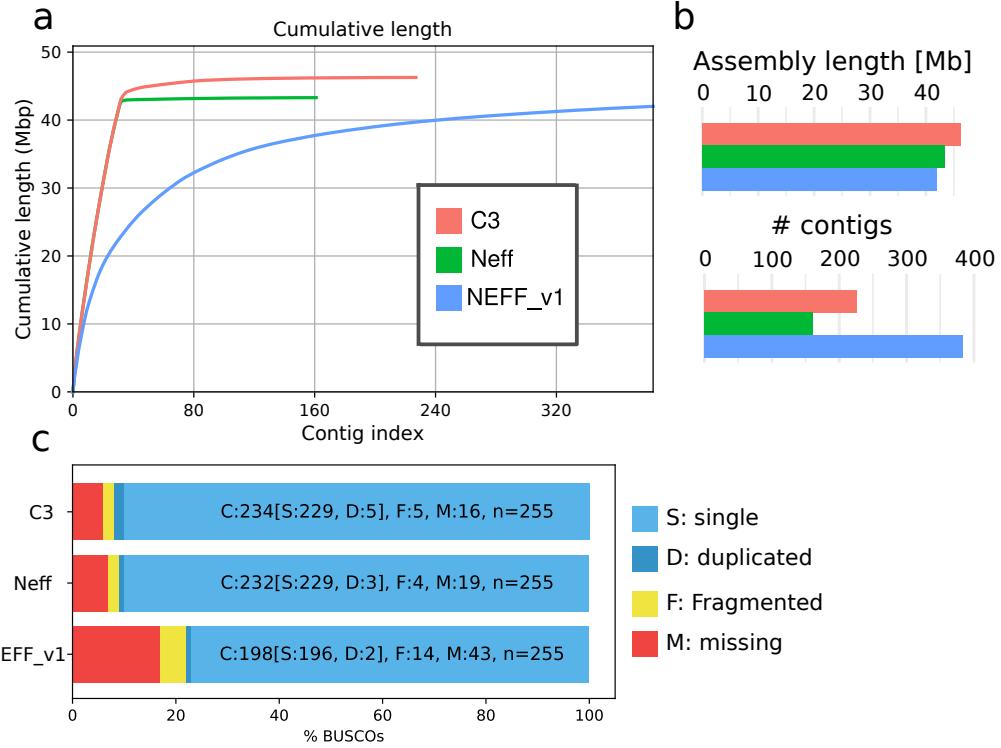


Figure 1: Assembly statistics for *A. castellanii*. Comparison genome assemblies for strains C3 and Neff, versus the previous NEFF-v1 genome assembly [24]. **a**, Cumulative length plot showing the relationship between number of contigs (largest to smallest) and lenght of the assembly. **b**, General continuity metrics. **c**, BUSCO statistics showing the status of universal single copy orthologs in eukaryotes for each assembly.

of both the C3 and Neff strains to generate genomewide contact maps. Visualising the Hi-C contact maps of both strains reveals that *A. castellanii* chromosomes are well resolved in our assembly (Fig. 2). In Neff, the highest intensity contacts are concentrated on the main diagonal, suggesting an absence of large-scale misassemblies. On the other hand, the C3 assembly retains a few misassembled blocks, mostly in the rDNA region where tandem repeats could not be resolved correctly. However, for both strains the genomewide contact maps reveal a grid-like pattern, with contact enrichment between chromosome extremities resulting in discrete dots. These contacts can be interpreted as a clustering of the telomeres, or subtelomeres, of the different chromosomes (Fig. 2). Additionally we found ~ 100 copies of 5S ribosomal DNA genes dispersed on most chromosomes for both strains, and 18S/28S genes show increased contacts with subtelomeres (Fig. 2).

In addition to large, trans- subtelomeric contacts, we also sought for intrachromosomal chromatin 3D structures in the contact maps using Chromosight, a pattern-detection program [31]. For both strains, the program pointed at arrays of chromatin loops along chromosomes, as well as boundaries separating chromatin domains (Fig. 2b). Most chromatin loops are regularly spaced, with a typical size of ~ 20 kb (Fig. 2c). The chromatin domains correspond to discrete squares along the diagonal (Fig. S3a). We overlapped all genes in the C3 strain with the domain borders we detected from Hi-C data and measured their base expression using RNAseq we generated from that strain (methods). We found that the genes overlapping domain boundaries are overall more highly expressed compared to the genome average (Fig. S2c). In addition, the analysis showed that gene expression is negatively correlated with the distance to the closest domain border (Fig. S2d). We performed the same comparison using chromatin loop anchors instead of domain borders. To a lesser extent, genes overlapping chromatin loops are also associated with a higher expression (Fig. S2a), although it is not correlated with the distance from the closest loop (Fig. S2b). Altogether, these results suggest that the chromatin structures observed in *cis* are both associated with gene expression, although the association between gene expression and chromatin loops basis is likely due to their co-localization with domain borders (Fig. S2e). These results are reminiscent of the organization of the chromosomes of microorganisms into small micro domains corresponding to expressed genes [32, 33].

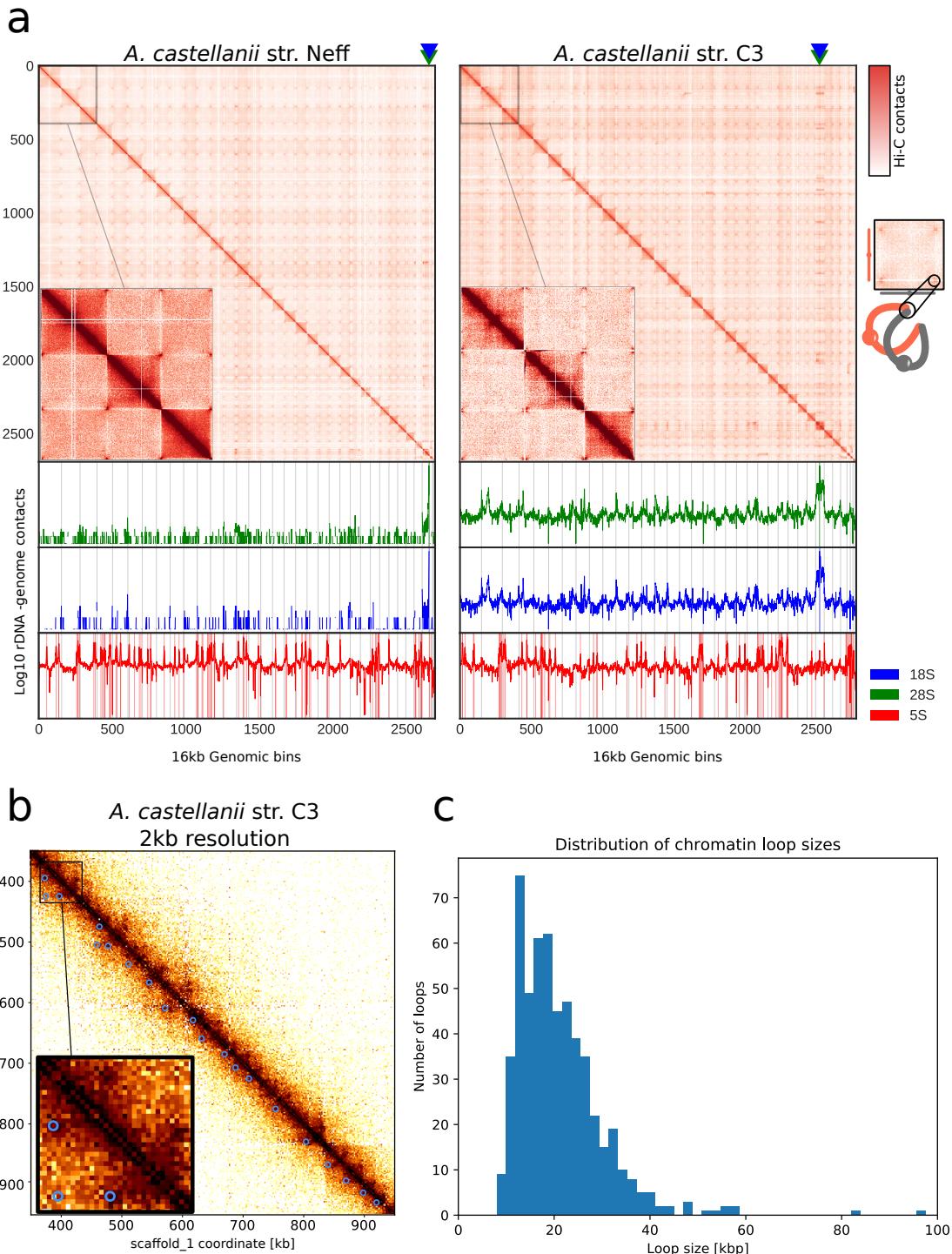


Figure 2: Spatial organisation of the *A. castellanii* genome. **a**, Top: Whole genome Hi-C contact maps of the Neff (left) and C3 (right) strains, with a magnification of the 3 largest scaffolds. The genome is divided into 16 kb bins, and each pixel represents the contact intensity between a pair of bins. Each scaffold is visible as a red square on the diagonal. In both strains, there is an enrichment of inter-scaffold contacts towards telomeres, suggesting a spatial clustering of telomeres, as shown on the model in the right margin. Bottom: 4C-like representation of spatial contacts between rDNA and the rest of the genome. Scaffolds are delimited by grey vertical lines. Contacts of all rDNAs are enriched towards telomere. The genomic position of 18S and 28S sequences are highlighted with triangles on the top panel and the occurrences of 8S rDNA sequences are shown with vertical red lines on the bottom panel. **b**, High resolution the contact map for a region of the C3 genome showing chromatin loops detected by Chromosight as blue circles. **c**, Size distribution of chromatin loops detected in the C3 strain.

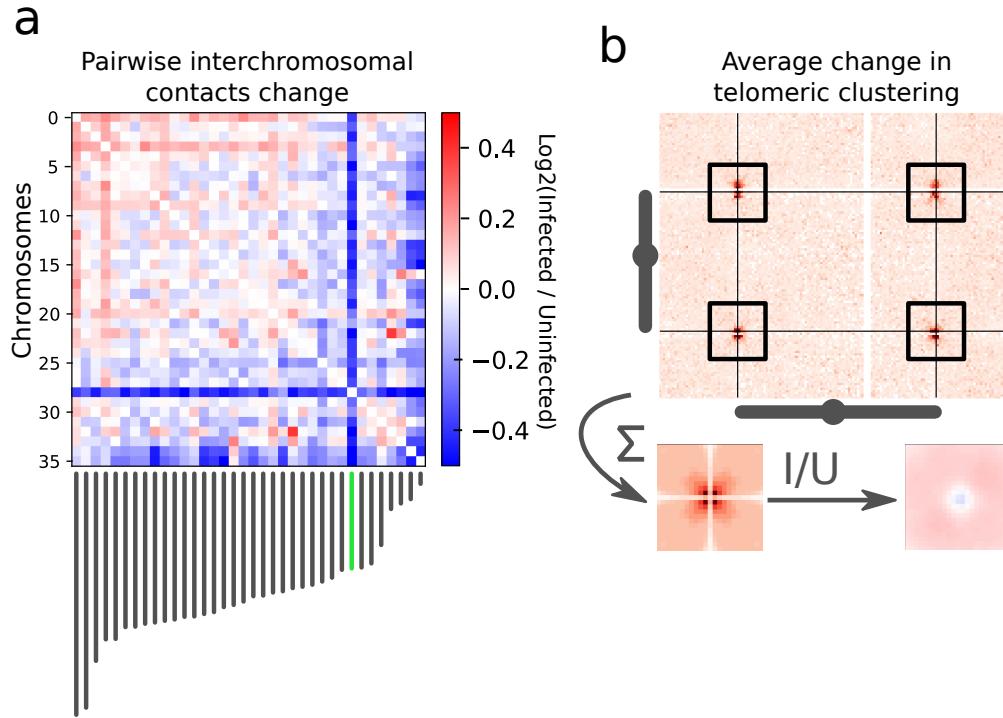


Figure 3: Changes in trans-chromosomal contacts between *A. castellanii* chromosomes following *L. pneumophila* infection. **a**, Average contact change during infection between each pair of chromosomes. Chromosome lengths are shown below the interaction matrix, with the chromosome bearing 18S and 18S rRNA subunits highlighted in green. **b**, Example inter-telomeric contacts between a pair of chromosomes. The average inter-telomeric contact profile generated from all pairs of chromosomes is shown as a pileup. The ratio between the infected (I) and uninfected (U) profiles is shown as a ratio (right).

L. pneumophila infection induces chromatin loop changes enriched in Rho GTPase, phosphorelay signal transduction, protein catabolism and GPI biosynthesis in the genome of *A. castellanii*

Having generated near complete assemblies allowed us to tackle the question of whether there is an effect of *L. pneumophila* infection on the 3D folding and transcription of *A. castellanii* C3 strain genome. We harvested cultured *A. castellanii* cells before and 5 hours following infection by *L. pneumophila* strain Paris [34] (Methods). The cells were processed using Hi-C and RNA-seq (Methods), and the resulting reads aligned against the reference genome to assess changes in the genome structure and the host transcription program, respectively. The RNAseq was performed in triplicates, and the Hi-C in duplicates (Methods). To measure changes in trans-chromosomal contacts, we merged replicates contact maps and applied the serpentine adaptative binning to improve the signal-to-noise ratio [35]. We then computed average interactions between each pair of chromosomes before and after infection. For each pair of chromosomes, we then used the log ratio of infected over uninfected average contacts. Following infection a global decrease in trans-subtelomeric contacts was observed, suggesting a slight de-clustering of chromosome ends (Fig. 3b). In addition, the scaffold bearing 18S and 28S rRNA (scaffold_29) genes, as well as two other small scaffolds (35 and 36) displayed weaker interactions with other scaffolds during infection (Fig. 3a).

We then assessed whether the behavior of cis-contacts changes during infection. First, we computed the average contact frequencies according to genomic distance $p(s)$ (Methods), which is a convenient way to unveil variations in the compaction state of chromatin [36]. The $p(s)$ curves show a global increase in long range contacts following infection (Fig. S4b). The strengths of chromatin loops and domain borders were quantified using Chromosight [31], before and 5h after infection. However, no significant average increase or decrease in the intensity of these structures (Fig. S4a) was identified when computed over the whole genome. To focus on infection-dependent chromatin structures, we filtered the top 20% detected patterns showing the strongest change in Chromosight score during infection (either appearing or disappearing). We performed a GO term enrichment analysis for genes associated with infection-dependent chromatin loops (methods). A significant enrichment for Rho GTPase and phosphorelay signal transduction, protein catabolism and GPI biosynthesis was found (Fig. S6a). Strongest loop changes were associated with genes including Rho GTPase, GOLD and SET domains as well as genes containing leucine-rich repeats and ankyrin repeats (Fig. S7).

We followed the same procedure for domain borders and found that genes associated with infection-dependent domain borders were significantly enriched in amino acid transport, cyclic nucleotide biosynthetic process, protein modification and deubiquitination (Fig. S6a). The presence of several other enriched metabolism-related terms is consistent with the fact that borders are generally associated with highly transcribed genes [37].

Using the *A. castellanii* RNAseq data after infection with *L. pneumophila*, we revealed that the expression of genes was globally impacted at 5h post infection compared to uninfected cells (Fig. S5a). To investigate the relationship between this change in gene expression and chromatin structures, we assigned the closest domain border to each gene and compared their expression and border score changes during infection. For the majority of genes, we found the border intensity to be decorrelated from gene expression changes (Fig. S5b). Only genes undergoing extreme expression changes during infection reflected changes in associated borders (Fig. S5c). This suggests that insulation domains in *A. castellanii* have a lesser importance in dictating gene expression programs than in mammals.

Recently, Li et al. [21] investigated gene expression changes at 3, 8, 16 and 24h after infection of *A. castellanii* Neff by *L. pneumophila*. They also reported a globally disrupted transcriptome at 8h compared to cells infected with $\Delta dotA$ mutant bacteria that are deficient of the essential type IV secretion system. To further validate our finding that chromatin domains are not units of regulation in *A. castellanii*, we used these expression results and lifted over the gene annotations to our C3 assembly using liftoff [38]. This allowed us to compute the co-expression between gene pairs during infection (i.e. expression correlation). We found that gene pairs sharing the same chromatin domain did not have a higher co-expression than gene pairs from different domains at similar genomic distances (Fig. S3).

Discussion

Generation, analysis and comparison of the genome sequences of two *A. castellanii* strains revealed a heterogeneous coverage across scaffolds which is consistent with previous findings that *A. castellanii* has a high but variable ploidy of approximately 25n. [39]. Considering the biology of *A. castellanii*, with suspected amitosis [40] and probable aneuploidies, a dispersion of 5S ribosomal DNA across all chromosomes would ensure a consistent copy number of 5S rDNA in daughter cells.

It was previously estimated that *A. castellanii* has 24 copies of the rDNA genes per haploid genome [41], but here we report 4 times more copies than previously thought in both strains. The decrease in interchromosomal contacts with rDNA-containing scaffold could reflect an alteration in the nucleolus structure. This could be caused by a global increase in translational activity during infection, which would be consistent with the global transcription shift observed in RNAseq.

At first glance, the contact maps show a clustering of subtelomeric regions, but do not display a Rabl conformation, where centromeres cluster to the spindle-pole body [42]. However, the precise positions of centromeres will be needed to verify they do not co-localize with subtelomeric regions.

Infection of *A. castellanii* with *L. pneumophila* induced significant changes in chromatin loops and borders. Our analyses showed an enrichment in several interesting GO terms during infection, many of them consistent with known biological processes induced by *L. pneumophila* in amoeba and macrophage cells. Several enriched terms are related to cell cycle regulation, including mitotic cell cycle, cell cycle processes or cell cycle checkpoints (Fig. S6), which might be associated to recent results showing that *L. pneumophila* prevents proliferation of its natural host *A. castellanii* [43, 21]. Legionella-induced alteration of the host cell cycle might be related to the avoidance of cell cycle phases that restrict bacterial replication in human cells [44] or prevention of amoebal proliferation, which has been proposed to increase the efficiency of single amoeba to feed on bacterial preys [45]. Several other GO terms enriched during infection are related to host cell organelles, such as organelle assembly, microtubule cytoskeleton organization, protein localization to endoplasmic reticulum, mitochondrion organization, electron transport chain or mitochondrial respiratory chain complexes (Fig. S6). It is well known that *L. pneumophila* hijacks host organelles such as the cytoskeleton, the endoplasmic reticulum or mitochondria in both hosts, amoeba or macrophages, during infection [46, 47, 48, 49]. Indeed, mitochondrial respiration and electron transport chain complexes were recently shown to be altered in macrophages during *L. pneumophila* infection [47, 50]. Other enriched GO terms are also related to changes in the general metabolism of the host, such as biosynthetic and catabolic processes, including nucleotide and nucleoside synthesis, lipid metabolism, or transport of amino acids or metal ions. To replicate intracellularly, *L. pneumophila* acquires all its nutrient from the cytoplasm of the host cell. Therefore, it is thought that bacteria-induced modulation of the host metabolism is key to establish a successful infection [51]. In summary, many of the GO terms associated to significant changes in chromatin loops and borders during infection reproduce the known biology of *Legionella* infection, suggesting that chromosomal rearrangements might be behind many of the observed changes in host cells during infection.

It was previously shown that *L. pneumophila* infection halts the host cell division and is associated with a decrease of mRNA of the *A. castellanii* CDC2b gene [43]. The large scale 3D changes we observed in chromatin compaction (Fig. S4b) and interchromosomal contacts (Fig. 3) are reminiscent of cell cycle changes in yeast and could suggest that the bacterium stops the host's cell cycle at a specific checkpoint.

We identified array of regularly spaced chromatin loop of approximately 20kb in size. This is also the range of the chromatin loops observed in *S. cerevisiae* during the G2/M stage [52]. This similarity in terms of regularity and size suggests that chromatin loops in *A. castellanii* may serve a similar purpose of chromosome compaction for cell division as in yeast. Our finding that DNA loops anchors overlap highly expressed genes is also concordant with observations made in yeast or other species [53], and result presumably from their role in blocking processing SMC complexes [54].

Unlike previously shown in Drosophila [55], we did not find and increase in co-expression for genes sharing the same contact domain in *A. castellanii*. This suggests chromatin domains may be caused by highly transcribed genes, and do not act as units of regulation as in other multicellular eukaryotes.

Methods

Strains and growth conditions

Infection timecourse

A. castellanii strain Neff and C3 were grown on amoeba culture medium (2% Bacto Tryptone, 0.1% sodium citrate, 0.1% yeast extract), supplemented with 0.1 M glucose, 0.1 mM CaCl₂, 2.5 mM Kh₂PO₄, 4 mM MgSO₄, 2.5 mM Na₂HPO₄, 0.05 mM Fe₄O₂₁P₆ at 20°C. *L. pneumophila* strain Paris was grown for 3 days on N-(2-acetamido)-2-aminoethanesulfonic acid (ACES)-buffered charcoal-yeast (BCYE) extract agar, at 37 °C. Infection of *A. castellanii* with *L. pneumophila* was performed using MOI 10 during 5h in infection medium (0.5% sodium citrate supplemented with 0.1 mM CaCl₂, 2.5 mM Kh₂PO₄, 4 mM MgSO₄, 2.5 mM Na₂HPO₄, 0.05 mM Fe₄O₂₁P₆ at 20°C. At 5h post-infection, amoeba were collected in a 15 mL tube, pelleted by centrifugation at 300 g for 10 minutes and washed twice in PBS, then crosslinked in 3% formaldehyde during 20 minutes at room temperature (RT) with gentle shaking. Then, 2.5 M glycine was added to reach a final concentration of 0.125 M during 20 minutes, centrifuged, washed, and pellets were stored at -80 °C until DNA extraction.

DNA was extracted using the QIAGEN Blood and Cell Culture DNA Kit (Qiagen) following the specific recommendations detailed by Nanopore in his info sheet entitled "High molecular weight gDNA extraction from cell lines (2018)" in order to minimize DNA fragmentation by mechanical constraints.

Library preparations

Hi-C

Hi-C libraries were prepared according to the Arima protocol using only the DpnII enzyme. Libraries were sequenced at 2x35bp on an Illumina NextSeq machine.

Shotgun

Shotgun libraries were sequenced by Novogene at 2x150bp on an Illumina Novaseq machine.

RNA-seq

RNAseq libraries were prepared using the stranded mRNA Truseq kit from Illumina and sequenced in single-end mode at 1x150bp on an Illumina NextSeq machine.

Nanopore sequencing

A. castellanii from str. Neff and C3 was extracted according to the Nanopore protocol for high molecular weight gDNA extraction from cell lines. Nanopore libraries were prepared with the ligation sequencing kit LSKQ109, flowcell model MIN106D R9. Basecalling was performed using Guppy v2.3.1-1.

Genome assembly

Nanopore reads were filtered using `filtlong v0.2.0` with default parameters to keep the best 80% reads according to length and quality. Illumina shotgun libraries were used as reference for the filtering. A *de-novo* assembly was generated from the raw (filtered) Nanopore long reads using `flye v2.3.6` with 3 iterations. The resulting assembly was polished using both Nanopore and Illumina reads with `HyPo v1.0.1`.

Contigs from the polished assembly bearing more than 60% of their sequence or 51% identity to the mitochondrial sequence from the NEFF_v1 assembly were separated from the rest of the assembly to prevent inclusion of mitochondrial contigs into the nuclear genome during scaffolding.

Polished nuclear contigs were scaffolded with Hi-C reads using `instagraal v0.1.2` with default parameters. Instagraal-polish was then used to fix potential errors introduced by the scaffolding procedure. Mitotic contigs were then added at the end of the scaffolded assembly and the final assembly was polished with the Illumina shotgun library using 2 rounds of `pilon` polishing. The resulting assembly was edited manually to remove spurious insertion of mitochondrial contigs in the scaffold and other contaminants. The final assembly was polished again using `pilon` with Rcorrector-corrected reads [56].

`Minimap2 v2.17` was used for all long reads alignments, and `bowtie2 v2.3.4.1` for short reads alignments.

Genome annotation

The Neff and C3 assemblies were annotated ...

Functional annotations were added using `funannotate v1.5.3`. Repeated sequences were masked using `repeatmasker`. Predicted proteins were fed to `Interproscan v5.22`, `Phobius v1.7.1` and `EggNOG-mapper v2.0.0` were used to generate functional annotations. Ribosomal DNA genes were annotated separately using `RNAmmer v1.2` with `HMMER 2.3.2`.

The `funannotate`-based script "func_annot_from_gene_models.sh" used to add functional annotations to existing gene models is provided in the zenodo record xx and on the associated github repository as described in the code availability section.

Comparative analyses

Proteomes of 11 other amoeba species and 3 intracellular bacteria associated to *A. castellanii* were retrieved from NCBI. Redundant sequences in each proteomes with more than 95% identity were filtered out using `CDHit v4.8.1`. The protein sequences were fed to `orthofinder v2.3.3` to obtain orthogroups of proteins, and a matrix of presence-absence was built to identify potential HGT events present in *A. castellanii* and intracellular bacteria, but not other amoeba species.

The pipeline for comparative genomics analyses is available on Github at: ...

To compute proportion of substituted positions in aligned segments between C3 and Neff strains, the two genomes were aligned using `minimap2` with the `asm20` preset and `-c` flag. The gap-excluded sequence divergence (mismatches / (matches + mismatches) was then computed in each primary alignment and the average of divergences (weighted by segment lengths) was computed.

Hi-C analyses

Reads were aligned with `bowtie2 v2.4.1` and Hi-C matrices were generated using `hicstuff v3.0.1` (<https://www.github.com/koszullab/hicstuff>). For all comparative analyses, matrices were downsampled to the same number of contacts using `cooltools` (<https://www.github.com/mirnylab/cooltools>) and balancing normalization was performed using the ICE algorithm [57]. Loops and domain borders were detected using `Chromosight v1.6.1` [31] using the merged replicates at a resolution of 2 kbp. We measured the intensity changes during infection using `pareidolia v0.6.1` (<https://www.github.com/koszullab/pareidolia>) on 3 pseudo replicates generated by sampling the merged contact maps, as described in [58]. This was done to account for contact coverage heterogeneity across replicates. The 20% threshold used to select differential patterns amounts to 1.2% false detections for loops and 2.3% for borders when comparing pseudo-replicates from the same condition.

Code availability

The analyses are packaged into the following snakemake pipelines available on github.

- Hybrid genome assembly: https://github.com/cmdoret/Acastellanii_hybrid_assembly
- Functional annotation of *A. castellanii*: https://github.com/cmdoret/Acastellanii_genome_annotation
- Analyses of genomic features in *A. castellanii*: https://github.com/cmdoret/Acastellanii_genome_analysis
- Changes during infection by Legionella: https://github.com/cmdoret/Acastellanii_legionella_infection.

All aforementioned repositories have been linked to Zenodo record xx available at: xx

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Supplementary figures

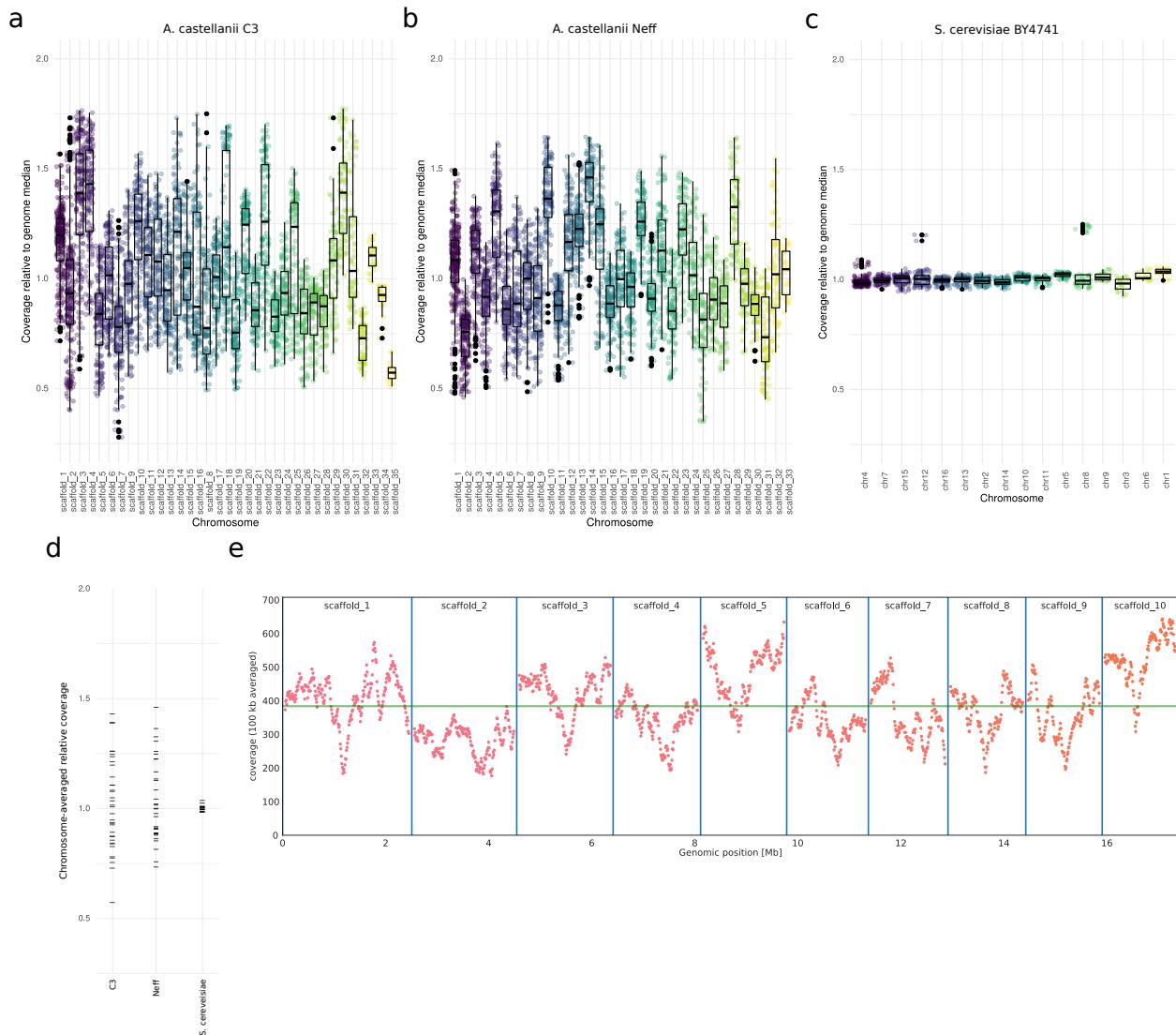


Figure S1: Coverage across scaffolds of *A. castellanii* compared to a known haploid. Distribution of mean Illumina shotgun coverage in 100kb sliding windows, normalized by the genome median, across **a** *A. castellanii* Neff and **b** C3 scaffolds compared to **c** asynchronous *Saccharomyces cerevisiae* strain BY4741, a known haploid. Variability of median coverage per chromosome for all three organisms. **e**, Sliding window coverage along the 10 largest scaffolds of *A. castellanii* Neff, with the horizontal green line showing genome median coverage. For *S. cerevisiae*, library SRR1569870 was used.

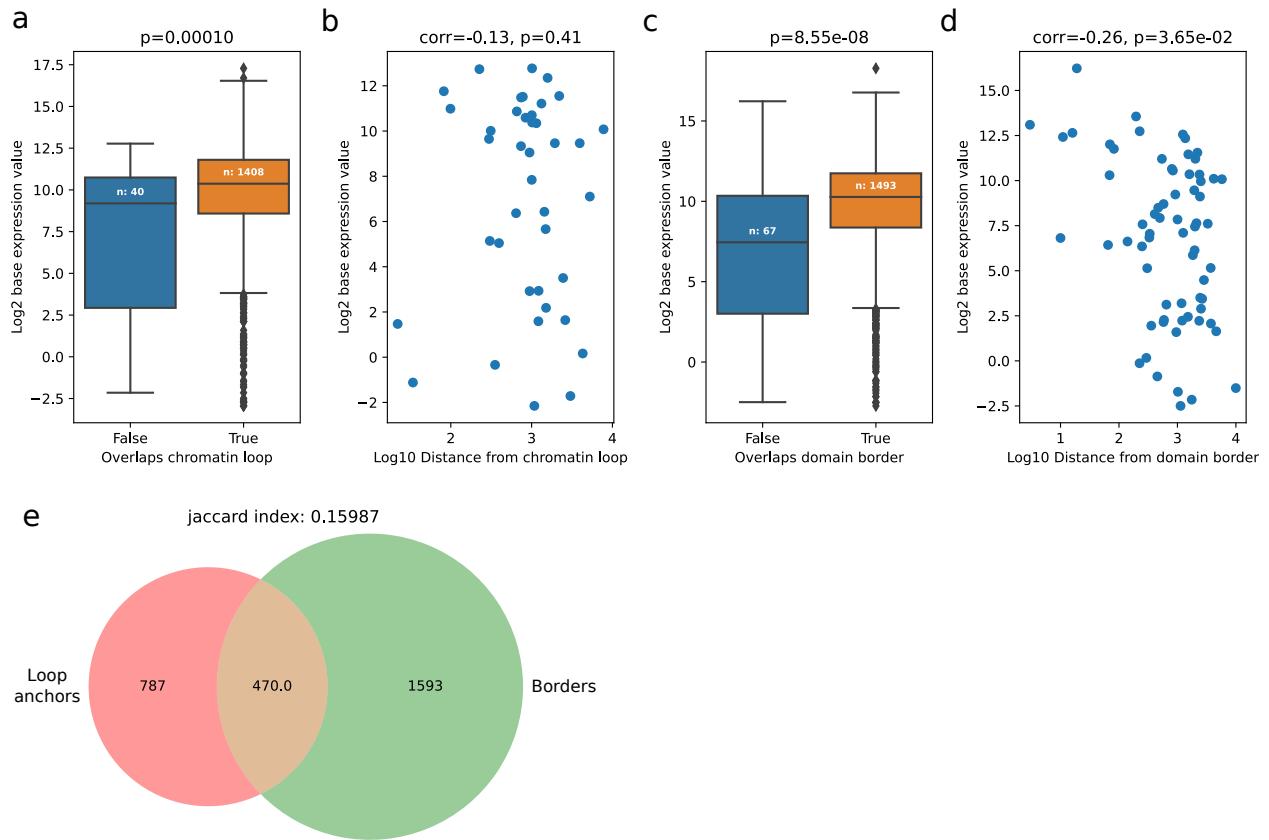


Figure S2: Gene expression according to position relative to chromatin structures. Gene expression versus **a**, overlap status with chromatin loops and **b**, distance to closest loop. Gene expression versus **c**, overlap status with domain borders and **d**, distance to closest border. P-values reported for overlap comparisons are obtained using Mann-Whitney U test, correlation coefficients and associated p-values are computed using Spearman's correlation test. **e**, Overlap between chromatin loop anchors and domain borders represented as a Venn diagram.

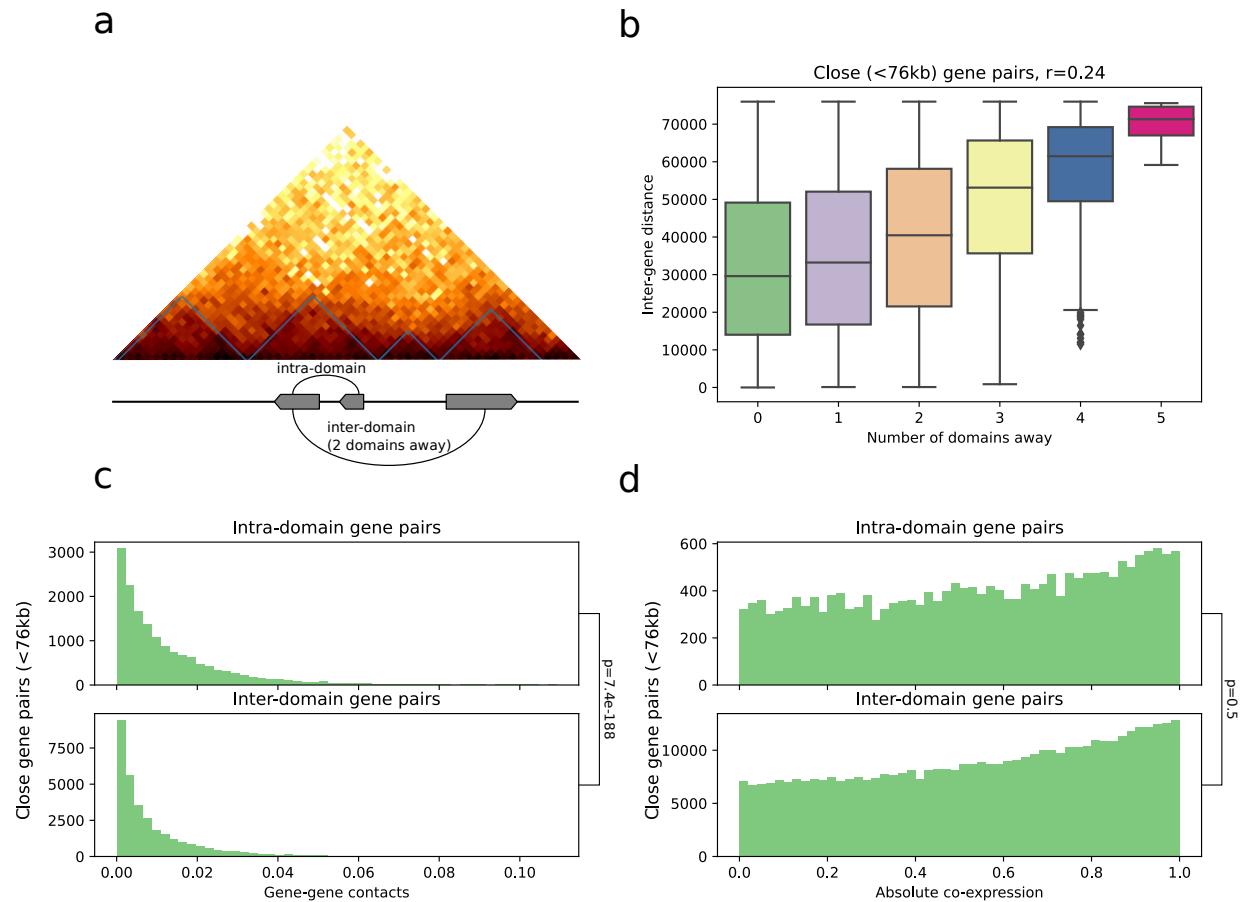


Figure S3: Relationship between genes and insulation domains. **a**, Example domains detected by Chromosight in the C3 strain, with theoretical genes for demonstration. **b**, Relationship between inter-gene distance and number of domains separating them. **c**, Distribution of mean inter-gene contacts according to domain separation status. **d**, Distribution of gene-pairs co-expression according to domain separation status. For all panels, only gene pairs separated by less than the median domain size were selected.

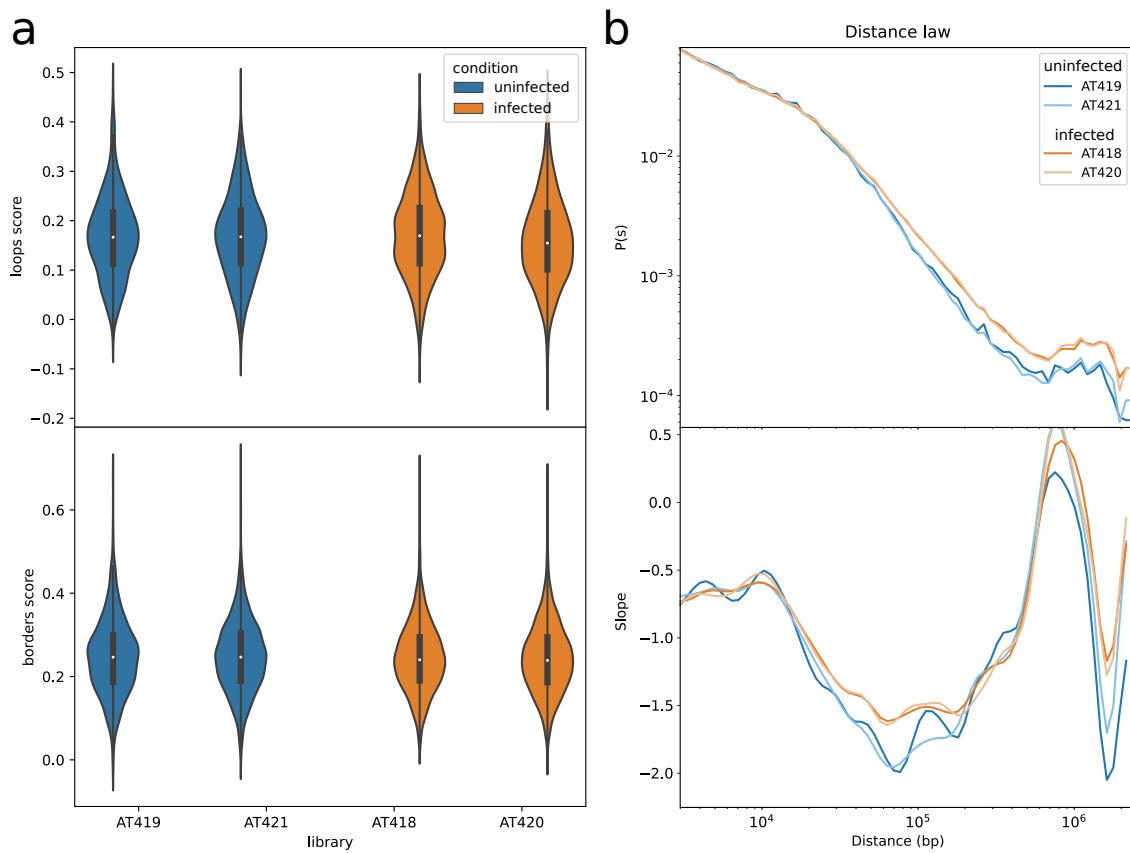


Figure S4: Global comparisons of infection Hi-C results between replicates. **a**, Distribution of Chromosight loops and borders scores for all 4 samples. **b**, Distance-contact decay function (denoted $P(s)$) and its slope.

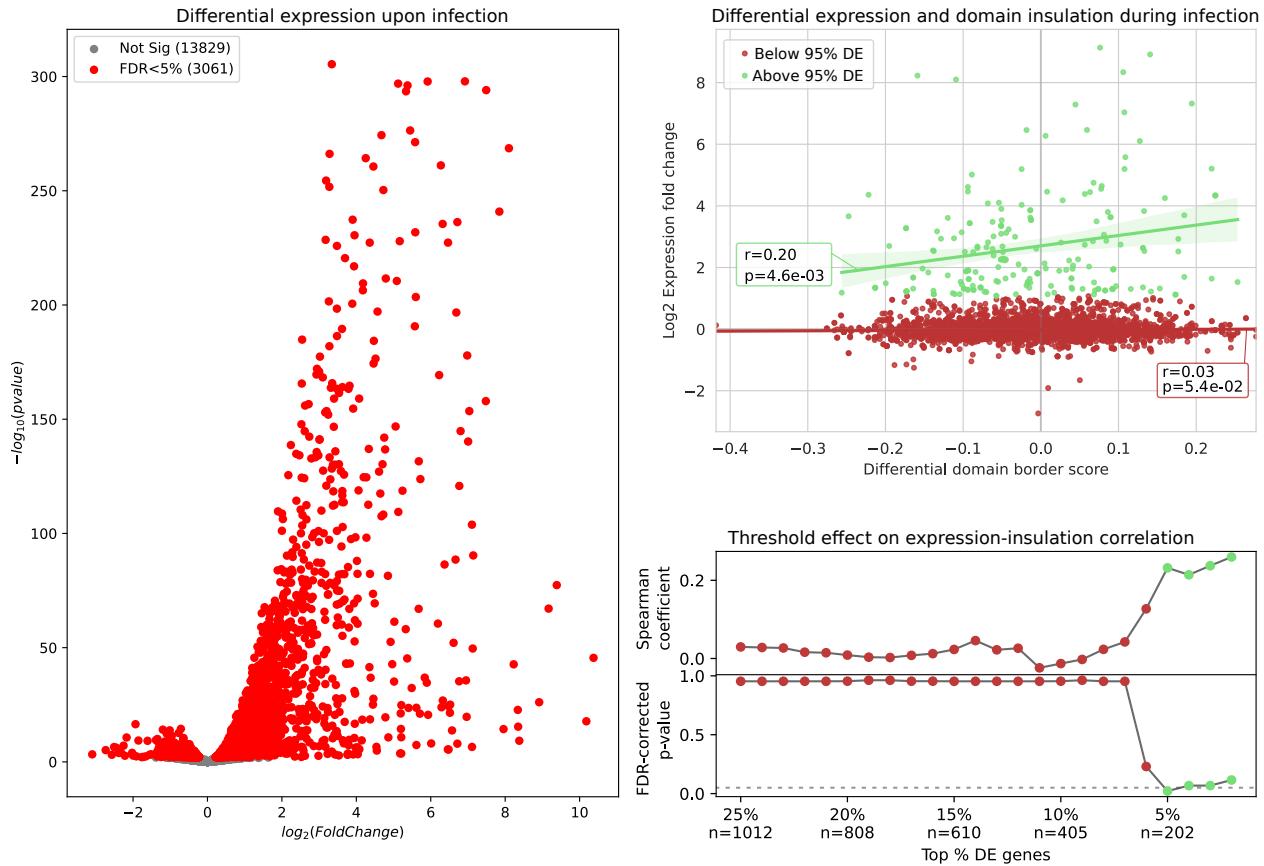


Figure S5: Relationship between differential expression and domain insulation during infection. **a**, Volcano plot showing differential gene expression (DE) of infected (5h p.i.) versus uninfected amoeba. Genes with significant corrected p-values (FDR<5%) are shown in red. **b**, Changes in gene expression and insulation strength of closest domain border during infection. Linear regression lines, Spearman correlation coefficients and associated p-values are shown separately for genes with extreme fold change values (95% quantile) and the rest. **c**, Spearman correlation coefficient between expression fold change and domain insulation change, and associated FDR-corrected p-values (FDR<5%) for different subsets of genes according to the threshold of extreme fold change. Values are colored according to the 95% threshold selected in b.

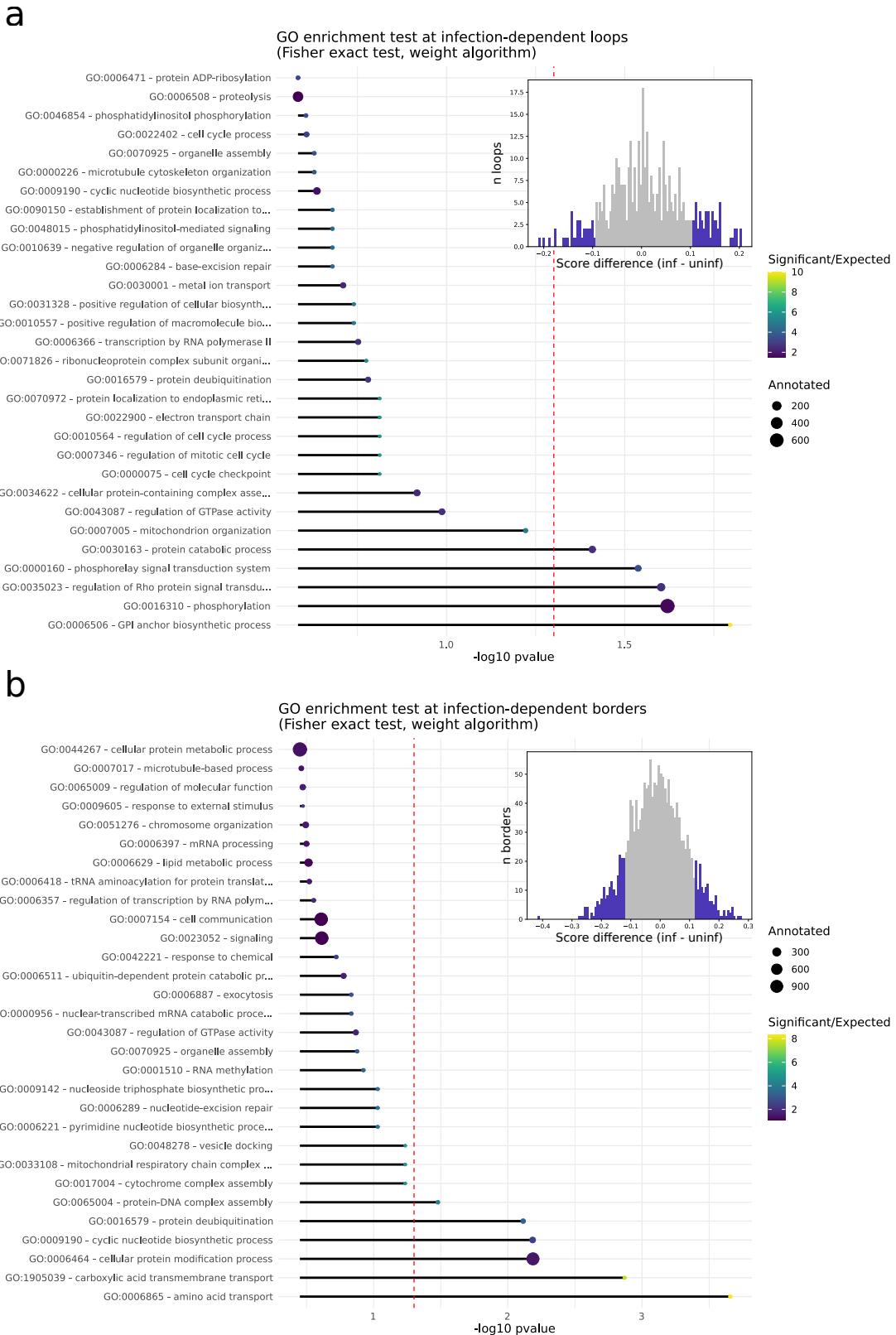


Figure S6: GO term enrichment test results for genes overlapping infection-dependent **a**, chromatin loops and **b**, domain borders. Histograms show the distribution of loop and border score changes during infection, with highlighted portions showing the 80% percentile threshold used to include genes in the GO enrichment test.

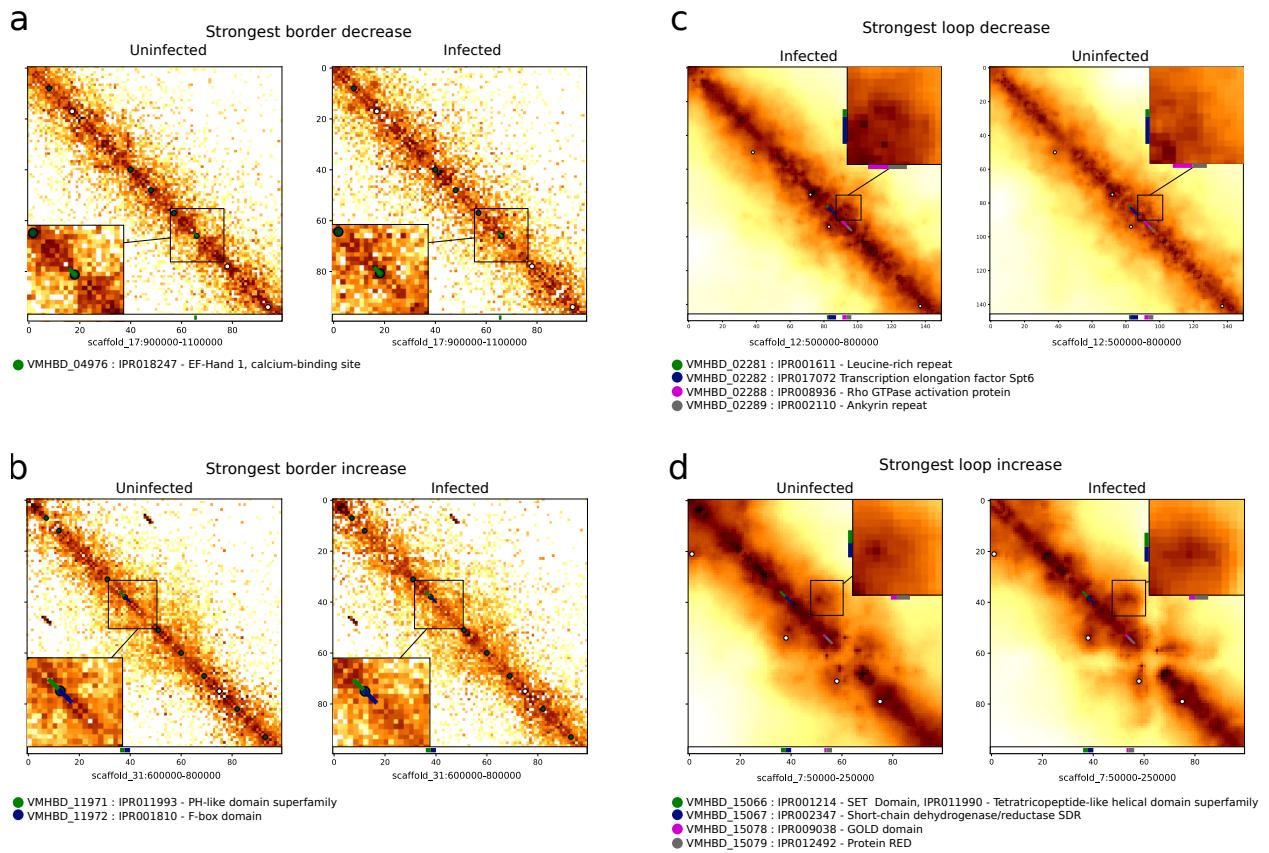


Figure S7: Hi-C zooms on strongest pattern changes during infection. Description of the closest genes are shown below each zoom. Balanced contact map zooms showing **a**, strongest border decrease and **b**, decrease. Serpentine-binned contact maps showing **c**, strongest loop decrease and **d**, increase.