GENOME WATCH

Unravelling the *Laverania*

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How did an ape-infecting *Plasmodium* species jump to a human host?

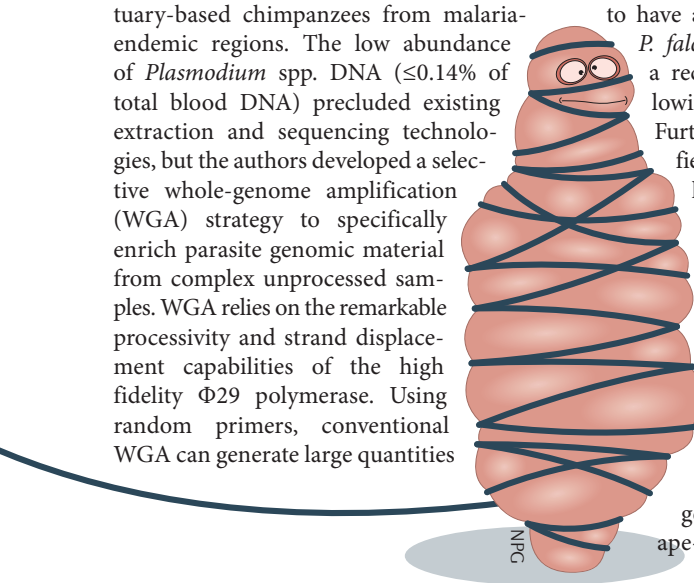
Plasmodium falciparum is evolutionarily distant from the other four *Plasmodium* species that are known to infect humans but is closely related to parasites that infect wild-living apes, with which it forms the *Laverania* subgenus. Each *Laverania* species seems to be restricted to a single ape host, even when different host species coexist in the same geographical region. However, significantly, *Laverania* host switching has occurred in the past, as *P. falciparum* emerged from an ancestral parasite that infected gorillas. To better understand the association of *Laverania* parasites with different ape hosts, a recent study developed a protocol for whole-genome sequencing of parasite DNA.

Detailed study of *Laverania* parasites has been limited by the endangered status of their ape hosts and an absence of *in vitro* culture systems. To circumvent these considerable ethical and logistical issues, Sundararaman *et al.*[1] used blood that was taken during routine health checks of sanctuary-based chimpanzees from malaria-endemic regions. The low abundance of *Plasmodium* spp. DNA (≤0.14% of total blood DNA) precluded existing extraction and sequencing technologies, but the authors developed a selective whole-genome amplification (WGA) strategy to specifically enrich parasite genomic material from complex unprocessed samples. WGA relies on the remarkable processivity and strand displacement capabilities of the high fidelity Φ29 polymerase. Using random primers, conventional WGA can generate large quantities of genomic DNA from tiny amounts of starting material. However, in complex mixtures of DNA, rare templates would be swamped and thus not amplified for downstream analysis. Two clever tricks were used to add selectivity to the WGA process: first, random primers were replaced with primers that targeted sequence motifs that are only common in *Plasmodium* spp. genomes; and, second, methylation-dependent restriction enzymes were used to specifically degrade host DNA. With these modifications, WGA was remarkably effective at selectively amplifying parasite DNA, as shown by Illumina MiSeq sequencing, which revealed a substantial enrichment of target DNA. Indeed, sufficient parasite-specific MiSeq reads (up to 89% of total reads) were generated to assemble draft genomes for two chimpanzee-infecting species, *Plasmodium reichenowi* and *Plasmodium gaboni*.

Analysis of the sequences that were generated using the selective WGA protocol provided valuable insights into the evolution and biology of *Laverania* species. For example, ape-infecting *Laverania* species were shown to have a greater genetic diversity than *P. falciparum*, which could point to a recent population bottleneck following the change in host species. Furthermore, adaptations were identified in the machinery that mediates host–parasite interactions, such as the acquisition by horizontal gene transfer of two essential invasion genes, which occurred in a gorilla parasite ancestral to *P. falciparum*, and a substantial *Laverania*-specific expansion of a gene family that is involved in erythrocyte remodelling.

An important outstanding question regarding the emergence of *P. falciparum* from an ape-infecting parasite is, what are the processes that control the host specificity of *Laverania* species? This complex issue may involve the interaction of host, parasite and vector factors. Makanga *et al.*[2] have recently investigated the effect of vector feeding preference on host specificity by surveying anopheline mosquitoes, which are vectors for *Laverania* parasites, in forests that are inhabited by wild apes. Although *Laverania* parasites could only be detected in samples from a relatively small number of anopheline mosquito species, PCR amplification of mitochondrial DNA established that chimpanzee-infecting and gorilla-infecting *Plasmodium* species were both present in the same mosquito species. This finding indicates that vector feeding preference is not responsible for the restriction of each *Laverania* species to a single host and that other mechanisms must instead prohibit host switching.

Understanding the zoonotic capacity of ape-infecting *Plasmodium* spp. parasites has clear implications for the development of effective strategies to control malaria. Comparative and functional genomics will be key to future research on the determinants of host specificity, and sequencing all *Laverania* genomes is therefore a priority. As such, selective WGA promises to be an effective and powerful tool to finally make *Laverania* parasites amenable to modern genetic analyses.



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2. Makanga, B. *et al.* Ape malaria transmission and potential for ape-to-human transfers in Africa. *Proc. Natl Acad. Sci. USA* **113**, 5329–5334 (2016).

Competing interests statement
The author declares no competing interests.