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Trends in Parasitology

Forum

Ongoing host-shift speciation in *Plasmodium simium*

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***Plasmodium simium*, a malaria parasite that infects platyrrhine monkeys and humans in the New World, is nearly identical to *Plasmodium vivax*. Recent genomic comparative analyses of these sister species have identified elevated divergence in a gene that may underlie *P. simium* adaptation to non-human primates during its gradual speciation process.**

A new simian malaria parasite

Flavio da Fonseca identified *P. simium* in 1951 while re-examining blood smears from an *Alouatta guariba clamitans* howler monkey (Figure 1A) caught one decade earlier in the Atlantic Forest remnants next to the city of São Paulo, Southeast Brazil [1]. At that time, *Plasmodium brasilianum* was the only malaria parasite known to naturally infect Neotropical monkeys. Fonseca described four major morphological differences between the new species and *P. brasilianum*: (i) the presence of prominent Schüffner dots in infected red blood cells; (ii) the increased size of infected red blood cells compared with uninfected cells; (iii) the presence of ≥16 merozoites in mature schizonts; and (iv) the rarity of band forms (Figure 1B–I). The new parasite closely resembled *P. vivax,* but its occurrence in a non-human primate prompted Fonseca to place *P. simium* in a separate species. Its host range was subsequently found to extend to woolly spider monkeys, capuchin monkeys, and titis.

Where did *P. simium* come from?

New malaria parasite species often originate from host shifts, when the ancestral species becomes established in new vertebrate hosts [2]. This appears to be the case for *P. simium*, which is genetically very similar to its sister species *P. vivax*, the most common cause of malaria in the Americas. By contrast, there is extensive genome-wide divergence between worldwide *P. vivax* from humans and *P. vivax*-like parasites from apes in Central Africa, estimated at 2.2% across core genes [3].

P. simium exhibits nearly threefold less nucleotide diversity (average number of pairwise differences per site $\pi = 1.63 \times 10^{-4}$) [4] than Amazonian *P. vivax* populations (π between 4.9×10^{-4} and 6.2×10^{-4}) [5], consistent with a very recent host shift. The total number of host-shift events remains undetermined. Because *P. simium* isolates from howler monkeys harbor either of the two different types (VK247 and VK210) of the *P. vivax* circumsporozoite protein gene, parasite transfer from donor hosts to howlers must have occurred at least twice [6]. Additional transfers may be postulated to account for its presence in other non-human primate species. The close genetic affinity between *P. vivax* populations from Mexico and South America and *P. simium* [4,7] points to the New World as the new species' birthplace (Box 1).

Back and forth between humans and monkeys

Anophelines must take bloodmeals at the canopy of the trees in order to transmit *P. simium* to arboreal monkeys, and members of the *Kerteszia* subgenus fulfill this prerequisite [8]. The primary vector of *P. simium*, *Anopheles cruzii,* breeds in water trapped by the leaf axils of epiphytic bromeliads, flowering plants that are particularly abundant in the Atlantic Forest [9]. It also feeds at the ground level and may transmit malaria to humans [8].

Simian parasites have long been suspected to cause sporadic human malaria cases in Brazil. In 1966, Leonidas Deane and colleagues provided the first evidence that *P. simium* remained infectious to humans [8], but molecular evidence of zoonotic *P. simium* transmission emerged only five decades later, with the finding of identical mitochondrial haplotypes shared between parasites of human and simian origin in different settings across South and Southeast Brazil [10–12]. The extensive similarity between the newly characterized nuclear genomes of *P. simium* isolates from humans and monkeys [4,7] further confirms that they are indeed part of the same parasite lineage. Indeed, *P. simium* isolates of simian and human origin, from sites >700 km apart, may be as closely related to each other as meiotic siblings [4].

Genomic signatures of adaptation following a host shift

There is no consensus regarding the level of genome-wide nucleotide divergence required to define new evolutionary lineages as separate malaria parasite species [2]. Under the key innovation model of recent speciation, sister species are expected to be genetically very similar except for a few genomic islands of elevated sequence divergence [13]. Recent comparative genomic analyses have identified a similar pairwise nucleotide divergence between *P. simium* from the Atlantic Coast and *P. vivax* from the Amazon (between-species $\pi = 4.92 \times 10^{-4}$) compared with the average divergence across *P. vivax* lineages that circulate in the Amazon Basin of Brazil (within-species $\pi = 4.93 \times 10^{-4}$) [4].

Changes in key erythrocyte invasion ligands are very common between genomes of the *Laverania* subgenus. One well-characterized example is the loss of

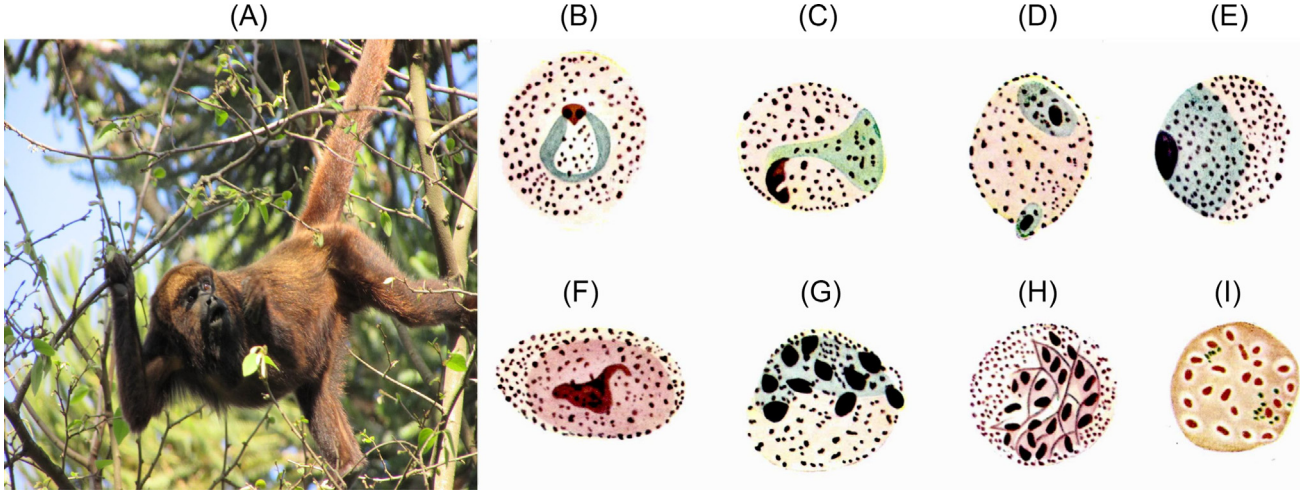

Figure 1. New host, new parasite. (A) Howler monkey (*Allouatta guariba clamitans*) photographed in Atlantic Forest remnants next to the city of São Paulo, Brazil (original by Ana Maria R.D. Duarte). (B–I) Original illustrations from Fonseca's 1951 article describing morphological characteristics of *Plasmodium simium* blood stages: (B) ring stage; (C) trophozoite resembling a band form; (D) double infection with young trophozoites within the same erythrocyte; (E) uninuclear stage resembling a macrogametocyte; (F) uninuclear stage; (G) young schizont with enlarged nuclei; (H) mature schizont with 23 nuclei; and (I) mature schizont with 22 nuclei (adapted from [1]).

EBA165, the red blood cell ligand of *Laverania* parasites that recognizes ape-specific sialic acids on erythrocytes, allowing *Plasmodium falciparum* to infect humans [14]. Genes encoding key erythrocyte invasion ligands of *P. simium* also display putative genomic signatures of adaptation to new vertebrate hosts, such as the deletion of >40% of the coding sequence of the *reticulocyte binding protein 2a* gene (*rbp2a*) in *P. simium* [4,7]. This drastic change might reduce RBP2a-mediated erythrocyte binding and favor alternative ligands for more efficient simian red blood cell infection. In addition, the *rbp2c* gene and the *rbp2d* pseudogene, members of the RBP family of *P. vivax,* are absent in the *P. simium* genome assembly [7].

Importantly, the genome of *P. vivax*-like isolates from chimpanzees of Central Africa comprises seemingly functional orthologs of *rbp2d*, *rbp2e*, and *rbp3,* which are pseudogenes in the human lineage of *P. vivax,* but these gains of RBP family members do not appear to enhance ape red blood cell specificity [3]. Whether *P. simium* binds to and invades human and simian erythrocytes with similar efficiency remains undetermined. The lack of a robust continuous *in vitro* culture protocol currently poses a major challenge to functional assays of red blood cell invasion in *P. vivax* and *P. simium.*

Concluding remarks

A major aim of parasitology is to understand how new parasite species evolve. *P. simium* may be seen as a regional *P. vivax* lineage at the very early stage of host-shift speciation along the Atlantic Coast of Brazil. The parasite survived human malaria elimination efforts between the 1950s and 1970s, thanks to its adaptation to a vast sylvatic reservoir, and is now geographically isolated from the *P. vivax* lineages that spread to the interior of the continent and infect exclusively humans [9].

As recently noted, 'because speciation is a gradual process, it could be argued that virtually all populations are in the process of speciation with a close or distant relative at any moment in time' [15]. We witness the birth of a zoonotic parasite species that faces the evolutionary trade-offs between the ability to infect a broad range of hosts, including humans and several non-human primate species, and the optimized adaptation to a particular vertebrate host.

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Declaration of interests

The authors declare no competing interests.

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Box 1. Geographic origins of *P. simium*

We hypothesize that a few *P. vivax* lineages from southern Europe and Africa that arrived on the Atlantic Coast of Brazil since the 1500s have evolved to infect *Anopheles cruzii* in nearby forested areas. The new vector facilitated the subsequent host shifts, from humans to arboreal monkey species – a unique example of malaria as a reverse zoonosis, a human infection that moved to sylvatic animals – and back to humans. Other *P. vivax* lineages introduced into the Americas spread out to the interior of the continent, dominated by anthropophilic vectors of the *Nyssorhynchus* subgenus, and founded the more diverse New World populations of *P. vivax* that infect humans (Figure I). Outside the Atlantic Forest, no vector appears to have successfully transferred *P. vivax* from humans to monkeys.

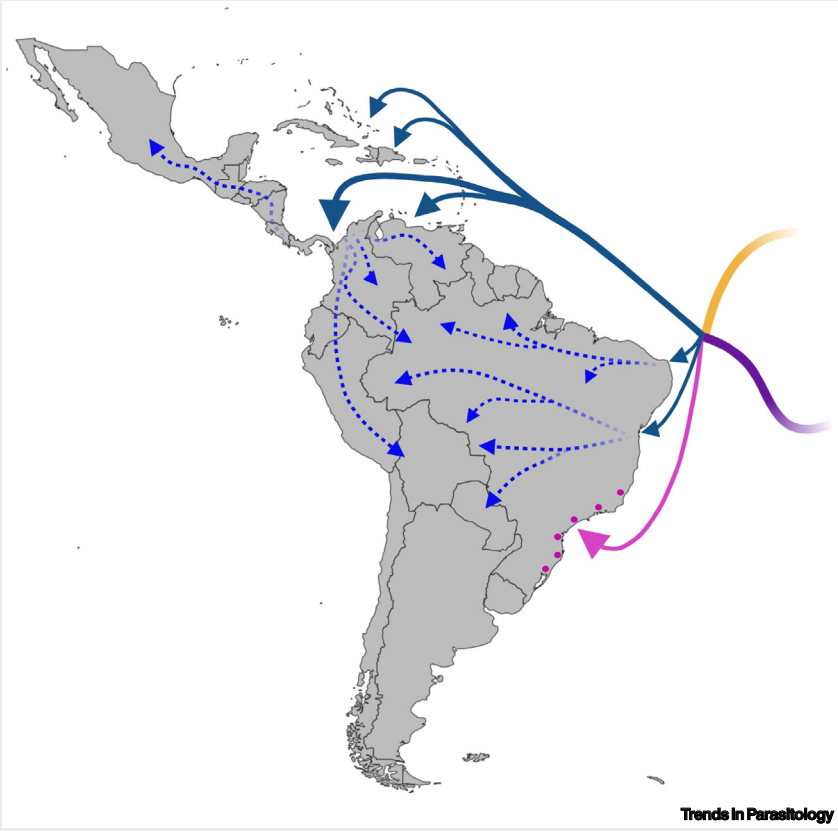

Figure I. Hypothetical routes of *Plasmodium vivax* introduction from Europe (yellow arrow tail) and Africa (purple arrow tail) into different regions of the Americas (blue or pink arrows). Parasites arriving in Southeast and South Brazil (pink arrows) adapted to *Kerteszia* vectors and New World monkeys and spread along the Atlantic Coast. Parasites arriving in areas where *Nyssorhynchus* vectors predominate spread to the entire continent and currently infect exclusively humans (broken blue arrows). Locations with documented simian or human *Plasmodium simium* infections along the Atlantic Coast are indicated with pink dots.

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