



Evolution of Host Specificity by Malaria Parasites through Altered Mechanisms Controlling Genome Maintenance

 Michelle C. Siao,^a Janus Borner,^{b,c} Susan L. Perkins,^{b*}  Kirk W. Deitsch,^d  Laura A. Kirkman^{d,e}

^aTri-Institutional MD-PhD Program, Weill Cornell Medical College, New York, New York, USA

^bSackler Institute for Comparative Genomics & Division of Invertebrate Zoology, American Museum of Natural History, New York, New York, USA

^cInstitute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Ulm, Germany

^dDepartment of Microbiology and Immunology, Weill Cornell Medical College, New York, New York, USA

^eDepartment of Internal Medicine, Division of Infectious Diseases, Weill Cornell Medical College, New York, New York, USA

ABSTRACT The protozoan parasites that cause malaria infect a wide variety of vertebrate hosts, including birds, reptiles, and mammals, and the evolutionary pressures inherent to the host-parasite relationship have profoundly shaped the genomes of both host and parasite. Here, we report that these selective pressures have resulted in unexpected alterations to one of the most basic aspects of eukaryotic biology, the maintenance of genome integrity through DNA repair. Malaria parasites that infect humans continuously generate genetic diversity within their antigen-encoding gene families through frequent ectopic recombination between gene family members, a process that is a crucial feature of the persistence of malaria globally. The continuous generation of antigen diversity ensures that different parasite isolates are antigenically distinct, thus preventing extensive cross-reactive immunity and enabling parasites to maintain stable transmission within human populations. However, the molecular basis of the recombination between gene family members is not well understood. Through computational analyses of the antigen-encoding, multicopy gene families of different *Plasmodium* species, we report the unexpected observation that malaria parasites that infect rodents do not display the same degree of antigen diversity as observed in *Plasmodium falciparum* and appear to undergo significantly less ectopic recombination. Using comparative genomics, we also identify key molecular components of the diversification process, thus shedding new light on how malaria parasites balance the maintenance of genome integrity with the requirement for continuous genetic diversification.

IMPORTANCE Malaria remains one of the most prevalent and deadly infectious diseases of the developing world, causing approximately 228 million clinical cases and nearly half a million deaths annually. The disease is caused by protozoan parasites of the genus *Plasmodium*, and of the five species capable of infecting humans, infections with *P. falciparum* are the most severe. In addition to the parasites that infect people, there are hundreds of additional species that infect birds, reptiles, and other mammals, each exquisitely evolved to meet the specific challenges inherent to survival within their respective hosts. By comparing the unique strategies that each species has evolved, key insights into host-parasite interactions can be gained, including discoveries regarding the pathogenesis of human disease. Here, we describe the surprising observation that closely related parasites with different hosts have evolved remarkably different methods for repairing their genomes. This observation has important implications for the ability of parasites to maintain chronic infections and for the development of host immunity.

KEYWORDS DNA repair, antigenic variation, evolution, malaria

Citation Siao MC, Borner J, Perkins SL, Deitsch KW, Kirkman LA. 2020. Evolution of host specificity by malaria parasites through altered mechanisms controlling genome maintenance. *mBio* 11:e03272-19. <https://doi.org/10.1128/mBio.03272-19>.

Editor Dominique Soldati-Favre, University of Geneva

Copyright © 2020 Siao et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Laura A. Kirkman, lak9015@med.cornell.edu.

* Present address: Susan L. Perkins, Department of Biology, The City College of New York, New York, New York, USA.

Received 12 December 2019

Accepted 18 February 2020

Published 17 March 2020

The coevolution of host and parasite, continuously adapting to each other for survival, is described by the Red Queen hypothesis in which “it takes all the running you can do, to keep in the same place” (1). This dynamic interaction is exemplified by malaria parasites, which are thought to have exerted the strongest known selective pressure on the human genome over the last 10,000 years (2), including numerous polymorphisms of red blood cell genes (3, 4). Different species of *Plasmodium* infect a broad range of vertebrate hosts, enabling a comparative analysis of adaptations particular to each specific host. Such comparisons have revealed unexpected changes in basic aspects of cell biology, from components of transcriptional machinery (5) to chromatin modifiers (6) and lipid metabolism (7–9), providing deep insights into the evolutionary pressures shaping these parasites, including aspects important for the human disease including pathogenesis, immune evasion, and transmission dynamics.

One unanticipated adaptation of all malaria parasites is the loss of classical nonhomologous end joining (cNHEJ), a fundamental mechanism responsible for repair of DNA double-strand breaks (DSBs). Malaria parasites depend almost entirely on homologous recombination (HR) to maintain genome integrity, despite spending most of their life cycle as haploid organisms and thus lacking the homologous chromosomes typically used for repair by HR (10). The loss of cNHEJ has been described in multiple parasitic lineages with several hypotheses put forward for how this may impact genome evolution and pathogenesis (11). We recently proposed a possible selective advantage for the loss of cNHEJ in the human malaria parasite *Plasmodium falciparum* (12). Within their vertebrate host, parasites avoid antibody-mediated clearance by varying the antigens that they express on the red cell surface, thus greatly extending the length of infections. This process, called antigenic variation, is dependent on extensive variability within the multigene families that encode these surface antigens (13). Furthermore, to enable reinfection of a previously infected host, different parasite strains must encode different repertoires of variant antigens. Thus, the capacity to generate new variants enables persistence within a host population even when most potential host organisms have developed clinical immunity, as is observed for *P. falciparum* infections in humans. The primary driving force for variant gene diversification is recombination between gene copies (14). In addition to sexual recombination, recombination can also occur between nonsyntenic genes (genes in different positions of the genome; this is also called ectopic recombination) during asexual replication when the parasites are haploid (15–17). This occurs when DNA DSBs arise in multigene family members. In the absence of NHEJ, such breaks must be repaired by HR using alternative members of the family from other positions in the genome as the template for repair (15, 16, 18). Thus, recombination between genes is not limited by genomic position, and diversification is greatly accelerated, resulting in an extraordinary degree of sequence diversity (19, 20). The selective pressure to continuously derive new variants through HR could provide a selective advantage for the loss of efficient NHEJ.

Given the importance of the parasite's ability to shuffle sequences between multigene family members, we were interested in defining the molecular basis underlying this process. We were therefore intrigued to find that, in stark contrast to the high degree of sequence divergence observed in *P. falciparum*, multigene family members in specific genomic positions were often nearly identical in different isolates of the rodent parasite *Plasmodium chabaudi* (Fig. 1A and B). A previous study similarly found that the multigene repertoires are often conserved between these two isolates (21), suggesting that recombination events are somehow more constrained in *P. chabaudi* than in *P. falciparum*. We were therefore curious if this observation could possibly provide clues to the underlying mechanism of diversification. For a more comprehensive study of recombination within these multigene families, we expanded our analysis to examine the genome assemblies of 16 *P. falciparum* isolates and five isolates from two subspecies of *P. chabaudi* (22). The availability of long-read sequencing of these genomes enabled our comparisons of gene variability with particular attention to syntenic genes, of multicopy gene families. For each gene with a mapped position, we systematically searched for the ortholog in the reference genome (3D7 for *P. falciparum*

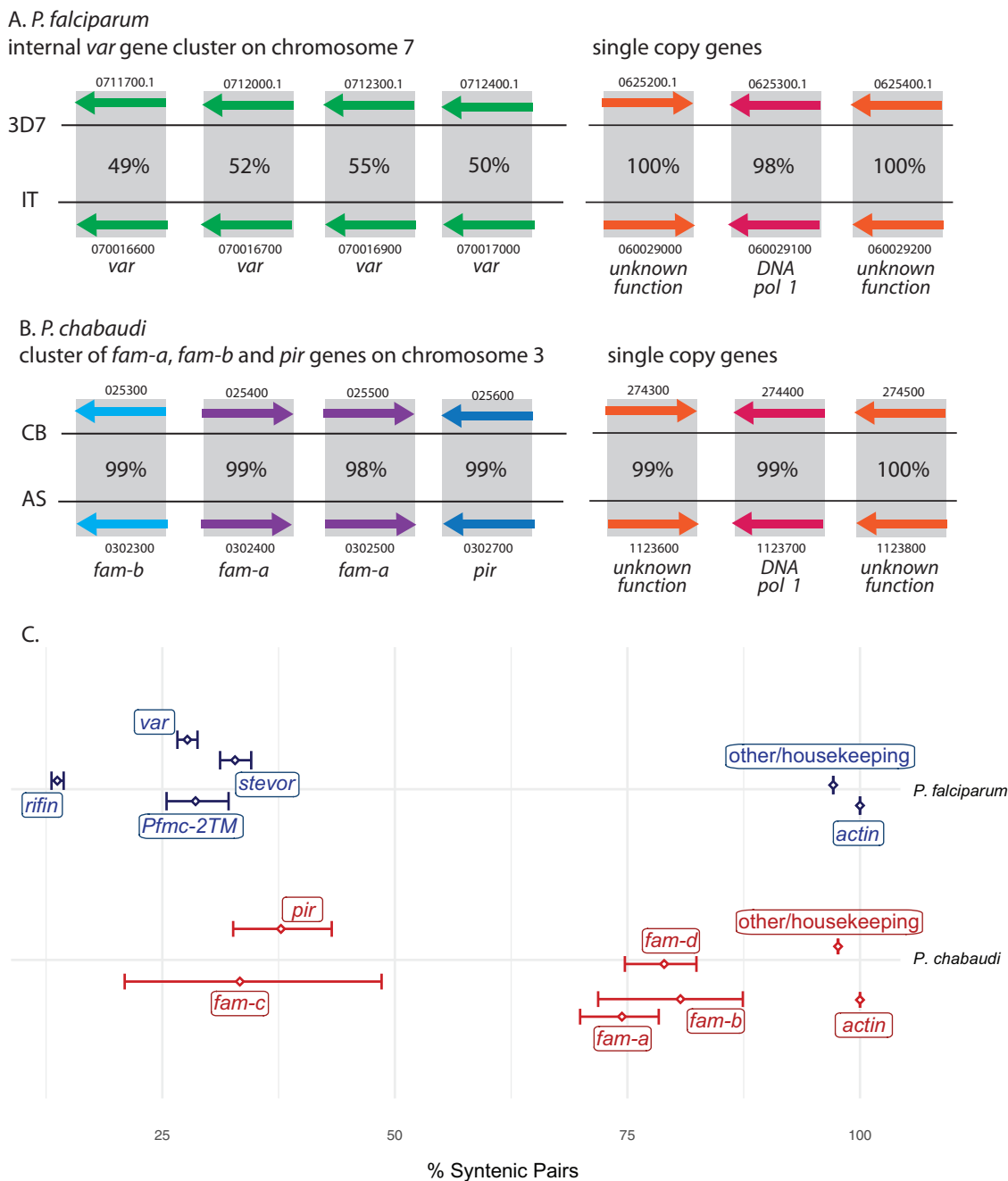


FIG 1 Comparison of variant antigen diversity in human and rodent malaria parasites. (A) (Left) Schematically shown are four members of the multicopy variant antigen *var* gene family of the human parasite *P. falciparum*. Genes from two geographical isolates (3D7 and IT) are shown from a syntenic region of chromosome 7, and the percentage of nucleotide identity between each gene is provided in the gray box enclosing each gene pair. Annotation numbers corresponding to the Eukaryotic Pathogen Genomics Database Resource (Release 45, EuPathDB, eupathdb.org [35]) are included above each arrow. (Right) A similar schematic shows the near-complete sequence identity observed for single-copy housekeeping genes. (B) A similar analysis as shown in panel A for two isolates (CB and AS) of the rodent parasite *P. chabaudi*. (Left) Members of the variant gene families *fam-a*, *fam-b*, and *pir*. (Right) Single-copy housekeeping genes. Sequence identities were calculated using Needleman-Wunsch alignment of two sequences (36). (C) Assessment of recombination within the multigene families. Individual genes from 15 independent isolates of *P. falciparum* (top, blue text) were compared to the 3D7 reference genome to identify the ortholog with the highest-scoring sequence alignment. For single-copy housekeeping genes, isolate-to-reference gene pairs were in the syntenic position of the genome nearly 100% of the time (right); such pairs for members of the *var*, *rifin*, *stevor*, and *Pfmc-2TM* variant gene families were seldom syntenic (left), indicating extensive recombination throughout these families. Similarly, 4 isolates of the rodent malaria parasite *P. chabaudi* (bottom, red text) were compared to the AS reference genome and demonstrated that a large majority of the *fam-a*, *fam-b*, and *fam-d* multigene family members maintained synteny, even across two subspecies. Diamonds represent percent synteny with 95% confidence intervals shown by error bars. See Text S1 in the supplemental material for details of sequence analysis.

and AS for *P. chabaudi*) with the highest-scoring alignment and then determined whether the paired sequences were located at comparable positions of their respective genomes. For single-copy housekeeping genes in both species, we found nearly universal synteny, as expected (Fig. 1C). For the multigene families *var*, *rifin*, *stevor*, and *Pfmc-2TM* of *P. falciparum* (23–25), gene pairs with the highest sequence identity were seldom syntenic (Fig. 1C, top), suggesting that recombination between nonsyntenic family members is common. In contrast, for the *P. chabaudi* isolates, the majority of *fam-a*, *fam-b*, and *fam-d* gene family members displayed the greatest sequence similarity to genes at the same genomic position (Fig. 1C, bottom), indicating relatively infrequent recombination between nonsyntenic genes. This pattern held even though the rodent parasite isolates examined represent two different subspecies of *P. chabaudi*. The trend was less pronounced for the *pir* gene family, which includes the *fam-c* subfamily and has been observed to display greater overall heterogeneity than the other variant gene families (26).

Given that both *P. chabaudi* and *P. falciparum* similarly lack cNHEJ and depend on HR for DNA double-strand break repair, an additional hypothesis is required to explain why members of the variant antigen gene families of rodent parasites appear to undergo significantly less extensive recombination among nonsyntenic genes. Generally among eukaryotes, translesion polymerases are required for efficient HR when the recombining sequences differ substantially (27), as they typically do when two non-identical variant antigen genes recombine. We observed that two translesion polymerases (orthologs of Rev1 and Pol ζ) and two accessory proteins (an SNF2 helicase and a RING finger/E3 ubiquitin ligase) are encoded in the genomes of most *Plasmodium* species and several related parasites spanning a range of vertebrate hosts (Fig. 2). Remarkably, all four of these genes are missing from the genomes of rodent malaria parasites, providing a likely explanation for the reduced degree of recombination and diversification that we detected within their multigene families. This led us to question what selective pressures could have resulted in the loss of this highly conserved DNA repair pathway specifically within the rodent malaria evolutionary lineage. Although many multigene family members of rodent parasites have been presumed to encode variant surface antigens as in primate malaria species, recent reports offer a different model in which different gene family members instead evolved distinct functions (28). If at least a subset of these genes perform distinct functions, these functions could be disrupted by recombination, thus favoring a mechanism to suppress recombination between nonsyntenic gene copies, such as through the observed loss of translesion polymerases. The reduction of antigenic diversity between isolates, as we found in rodent parasites, would presumably impair reinfection of hosts that have previously harbored an infection. However, several aspects of rodent parasite infections could influence the need for continuous diversification of antigen-encoding gene families, including the length and chronicity of infections, rates of transmission and the likelihood of reinfection, and the virulence of infections as well as the average life span of the hosts and their typical number of offspring. In addition, further analysis of rodent parasite genomes could reveal potential alternative mechanisms for DNA repair that perhaps partially compensate for the loss of translesion polymerases. For example, it is not clear how the *pir/fam-c* gene family members have acquired a much higher degree of diversity than the largely conserved *fam-a*, *fam-b*, and *fam-d* gene families, particularly considering that these families consist of similar numbers of genes and are located interspersed with one another within the parasite's genome.

Taken together, our observations suggest a key role for translesion polymerases in diversification of malaria parasite antigens. In model eukaryotes, these enzymes interface with nucleotide excision (29), base excision (30), and mismatch repair pathways (30), which are thought to be a major source of mutations leading to drug resistance in naturally circulating malaria parasites (31–34). Translesion polymerases may therefore play an underappreciated role in the continued threat of malaria to human health globally. This work underscores the power of comparative evolutionary studies to advance our understanding of parasite gene function and host-parasite interactions.

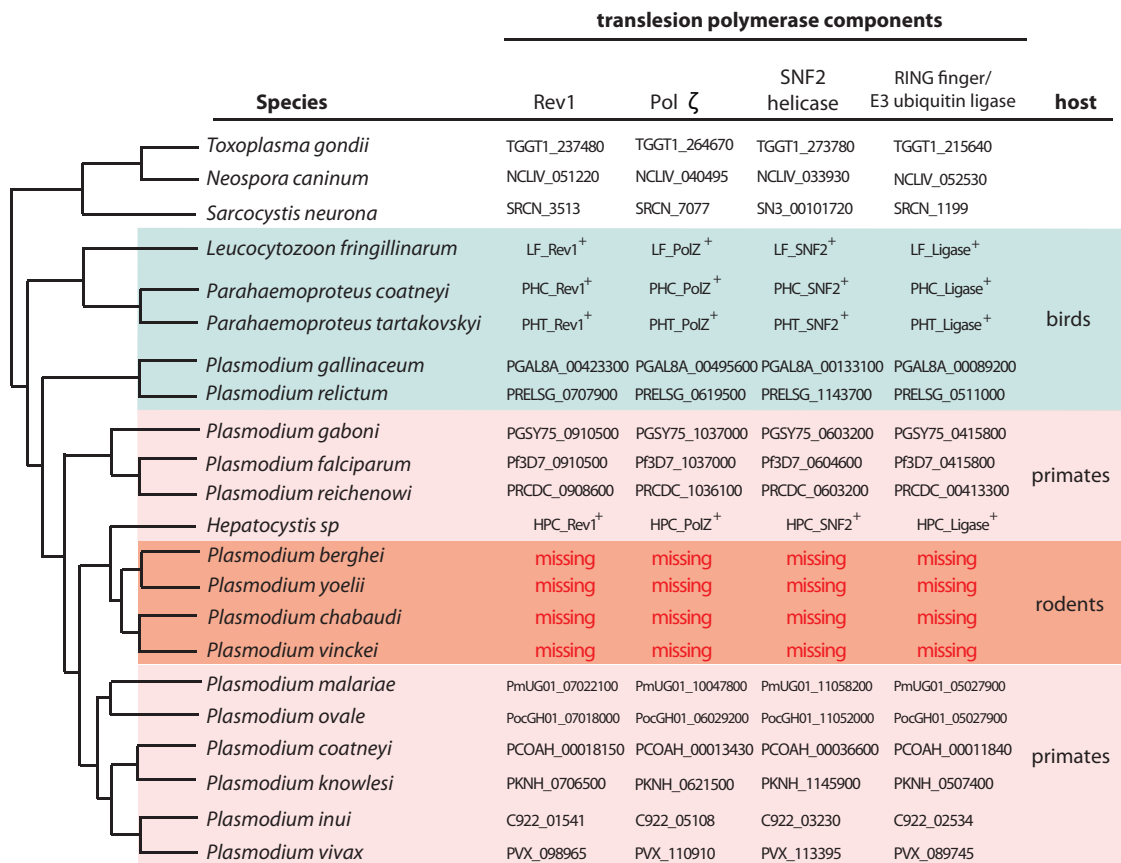


FIG 2 Phylogenetic tree of apicomplexan parasites based on the phylogeny of Galen et al. (37) showing the loss of translesion polymerases in different parasite lineages. *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis neurona* are parasites that do not possess large multigene families and do not undergo antigenic variation. Clades of parasites that infect red blood cells are highlighted with different-colored shading according to their vertebrate hosts. Rev1, Pol ζ, an SNF2 helicase, and a RING finger/E3 ubiquitin ligase are required for translesion polymerase activity. This analysis shows the loss of translesion polymerases in parasites that infect rodents. Gene annotation numbers are provided next to each species name for all species catalogued in the EuPathDB database (Release 45, EuPathDB, eupathdb.org [35]). Additional orthologous sequences were obtained from the fragmented genome assembly of *Parahaemoproteus tartakovskyi* (38), the transcriptome data sets of *Parahaemoproteus coatneyi* and *Leucocytozoon fringillinarum* (39), and sequence data of a *Hepaticystis* parasite that were mined from the transcriptome of a Ugandan red colobus monkey (40) using the ContamFinder pipeline (41). +, see Fig. S1 in the supplemental material for sequences and alignments of genes not previously annotated.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TEXT S1, PDF file, 0.03 MB.

FIG S1, PDF file, 0.2 MB.

REFERENCES

- Carroll L. 1900. Through the looking-glass and what Alice found there. WB Conkey Co, Chicago, IL.
- Kwiatkowski DP. 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* 77:171–192. <https://doi.org/10.1086/432519>.
- Feng Z, Smith DL, McKenzie FE, Levin SA. 2004. Coupling ecology and evolution: malaria and the S-gene across time scales. *Math Biosci* 189: 1–19. <https://doi.org/10.1016/j.mbs.2004.01.005>.
- Hedrick P. 2004. Estimation of relative fitnesses from relative risk data and the predicted future of haemoglobin alleles S and C. *J Evol Biol* 17:221–224. <https://doi.org/10.1046/j.1420-9101.2003.00635.x>.
- Kishore SP, Perkins SL, Templeton TJ, Deitsch KW. 2009. An unusual recent expansion of the C-terminal domain of RNA polymerase II in primate malaria parasites features a motif otherwise found only in mammalian polymerases. *J Mol Evol* 68:706–714. <https://doi.org/10.1007/s00239-009-9245-2>.
- Kishore SP, Stiller JW, Deitsch KW. 2013. Horizontal gene transfer of epigenetic machinery and evolution of parasitism in the malaria parasite *Plasmodium falciparum* and other apicomplexans. *BMC Evol Biol* 13:37. <https://doi.org/10.1186/1471-2148-13-37>.
- Witola WH, Ben MC. 2007. Choline induces transcriptional repression and proteasomal degradation of the malarial phosphoethanolamine methyltransferase. *Eukaryot Cell* 6:1618–1624. <https://doi.org/10.1128/EC.00229-07>.
- Déchamps S, Maynadier M, Wein S, Gannoun-Zaki L, Maréchal E, Vial HJ. 2010. Rodent and nonrodent malaria parasites differ in their phospholipid metabolic pathways. *J Lipid Res* 51:81–96. <https://doi.org/10.1194/jlr.M900166-JLR200>.

9. Dechamps S, Shastri S, Wengelnik K, Vial HJ. 2010. Glycerophospholipid acquisition in *Plasmodium*—a puzzling assembly of biosynthetic pathways. *Int J Parasitol* 40:1347–1365. <https://doi.org/10.1016/j.ijpara.2010.05.008>.
10. Kirkman LA, Lawrence EA, Deitsch KW. 2014. Malaria parasites utilize both homologous recombination and alternative end joining pathways to maintain genome integrity. *Nucleic Acids Res* 42:370–379. <https://doi.org/10.1093/nar/gkt881>.
11. Nenarokova A, Zahonova K, Krasilnikova M, Gahura O, McCulloch R, Zikova A, Yurchenko V, Lukes J. 2019. Causes and effects of loss of classical nonhomologous end joining pathway in parasitic eukaryotes. *mBio* 10:e01541-19. <https://doi.org/10.1128/mBio.01541-19>.
12. Zhang X, Alexander N, Leonardi I, Mason C, Kirkman LA, Deitsch KW. 2019. Rapid antigen diversification through mitotic recombination in the human malaria parasite *Plasmodium falciparum*. *PLoS Biol* 17:e3000271. <https://doi.org/10.1371/journal.pbio.3000271>.
13. Deitsch KW, Dzikowski R. 2017. Variant gene expression and antigenic variation by malaria parasites. *Annu Rev Microbiol* 71:625–641. <https://doi.org/10.1146/annurev-micro-090816-093841>.
14. Kraemer SM, Kyes SA, Aggarwal G, Springer AL, Nelson SO, Christodoulou Z, Smith LM, Wang W, Levin E, Newbold CI, Myler PJ, Smith JD. 2007. Patterns of gene recombination shape var gene repertoires in *Plasmodium falciparum*: comparisons of geographically diverse isolates. *BMC Genomics* 8:45. <https://doi.org/10.1186/1471-2164-8-45>.
15. Bopp SE, Manary MJ, Bright AT, Johnston GL, Dharia NV, Luna FL, McCormack S, Plouffe D, McNamara CW, Walker JR, Fidock DA, Denchi EL, Winzeler EA. 2013. Mitotic evolution of *Plasmodium falciparum* shows a stable core genome but recombination in antigen families. *PLoS Genet* 9:e1003293. <https://doi.org/10.1371/journal.pgen.1003293>.
16. Claessens A, Hamilton WL, Kekre M, Otto TD, Faizullabhoj A, Rayner JC, Kwiatkowski D. 2014. Generation of antigenic diversity in *Plasmodium falciparum* by structured rearrangement of Var genes during mitosis. *PLoS Genet* 10:e1004812. <https://doi.org/10.1371/journal.pgen.1004812>.
17. Claessens A, Harris LM, Stanojic S, Chappell L, Stanton A, Kuk N, Veneziano-Broccia P, Sterkers Y, Rayner JC, Merrick CJ. 2018. RecQ helicases in the malaria parasite *Plasmodium falciparum* affect genome stability, gene expression patterns and DNA replication dynamics. *PLoS Genet* 14:e1007490. <https://doi.org/10.1371/journal.pgen.1007490>.
18. Kirkman LA, Deitsch KW. 2014. Recombination and diversification of the variant antigen encoding genes in the malaria parasite *Plasmodium falciparum*. *Microbiol Spectr* 2(6). <https://doi.org/10.1128/microbiolspec.MDNA3-0022-2014>.
19. Otto TD, Bohme U, Sanders M, Reid A, Bruske EI, Duffy CW, Bull PC, Pearson RD, Abdi A, Dimonte S, Stewart LB, Campino S, Kekre M, Hamilton WL, Claessens A, Volkman SK, Ndiaye D, Amambua-Ngwa A, Diakite M, Fairhurst RM, Conway DJ, Franck M, Newbold CI, Berriman M. 2018. Long read assemblies of geographically dispersed *Plasmodium falciparum* isolates reveal highly structured subtelomeres. *Wellcome Open Res* 3:52. <https://doi.org/10.12688/wellcomeopenres.14571.1>.
20. Chen DS, Barry AE, Leliwa-Sytek A, Smith TA, Peterson I, Brown SM, Migot-Nabias F, Deloron P, Kortok MM, Marsh K, Daily JP, Ndiaye D, Sarr O, Mboup S, Day KP. 2011. A molecular epidemiological study of var gene diversity to characterize the reservoir of *Plasmodium falciparum* in humans in Africa. *PLoS One* 6:e16629. <https://doi.org/10.1371/journal.pone.0016629>.
21. Lin JW, Reid AJ, Cunningham D, Bohme U, Tumwine I, Keller-Mclaughlin S, Sanders M, Berriman M, Langhorne J. 2018. Genomic and transcriptomic comparisons of closely related malaria parasites differing in virulence and sequestration pattern. *Wellcome Open Res* 3:142. <https://doi.org/10.12688/wellcomeopenres.14797.2>.
22. Otto TD, Bohme U, Jackson AP, Hunt M, Franke-Fayard B, Hoeijmakers WA, Religa AA, Robertson L, Sanders M, Ogun SA, Cunningham D, Erhart A, Billker O, Khan SM, Stunnenberg HG, Langhorne J, Holder AA, Waters AP, Newbold CI, Pain A, Berriman M, Janse CJ. 2014. A comprehensive evaluation of rodent malaria parasite genomes and gene expression. *BMC Biol* 12:86. <https://doi.org/10.1186/s12915-014-0086-0>.
23. Lavstsen T, Salanti A, Jensen ATR, Arnot DE, Theander TG. 2003. Subgrouping of *Plasmodium falciparum* 3D7 var genes based on sequence analysis of coding and non-coding regions. *Malar J* 2:27. <https://doi.org/10.1186/1475-2875-2-27>.
24. Kraemer SM, Smith JD. 2003. Evidence for the importance of genetic structuring to the structural and functional specialization of the *Plasmodium falciparum* var gene family. *Mol Microbiol* 50:1527–1538. <https://doi.org/10.1046/j.1365-2958.2003.03814.x>.
25. Lavazec C, Sanyal S, Templeton TJ. 2006. Hypervariability within the Rfn1, Stevor and Pfmc-2TM superfamilies in *Plasmodium falciparum*. *Nucleic Acids Res* 34:6696–6707. <https://doi.org/10.1093/nar/gkl942>.
26. Frech C, Chen N. 2013. Variant surface antigens of malaria parasites: functional and evolutionary insights from comparative gene family classification and analysis. *BMC Genomics* 14:427. <https://doi.org/10.1186/1471-2164-14-427>.
27. McVey M, Khodaverdian VY, Meyer D, Cerqueira PG, Heyer WD. 2016. Eukaryotic DNA polymerases in homologous recombination. *Annu Rev Genet* 50:393–421. <https://doi.org/10.1146/annurev-genet-120215-035243>.
28. Fougere A, Jackson AP, Bechtsi DP, Braks JA, Annoura T, Fonager J, Spaccapelo R, Ramesar J, Chevalley-Maurel S, Klop O, van der Laan AM, Tanke HJ, Kocken CH, Pasini EM, Khan SM, Bohme U, van OC, Otto TD, Janse CJ, Franke-Fayard B. 2016. Variant exported blood-stage proteins encoded by *Plasmodium* multigene families are expressed in liver stages where they are exported into the parasitophorous vacuole. *PLoS Pathog* 12:e1005917. <https://doi.org/10.1371/journal.ppat.1005917>.
29. Enouï M, Jiricny J, Schärer OD. 2012. Repair of cisplatin-induced DNA interstrand crosslinks by a replication-independent pathway involving transcription-coupled repair and translesion synthesis. *Nucleic Acids Res* 40:8953–8964. <https://doi.org/10.1093/nar/gks670>.
30. Casali P, Pal Z, Xu Z, Zan H. 2006. DNA repair in antibody somatic hypermutation. *Trends Immunol* 27:313–321. <https://doi.org/10.1016/j.it.2006.05.001>.
31. Lee AH, Fidock DA. 2016. Evidence of a mild mutator phenotype in Cambodian *Plasmodium falciparum* malaria parasites. *PLoS One* 11:e0154166. <https://doi.org/10.1371/journal.pone.0154166>.
32. Castellini MA, Buguliskis JS, Casta LJ, Butz CE, Clark AB, Kunkel TA, Taraschi TF. 2011. Malaria drug resistance is associated with defective DNA mismatch repair. *Mol Biochem Parasitol* 177:143–147. <https://doi.org/10.1016/j.molbiopara.2011.02.004>.
33. Bethke L, Thomas S, Walker K, Lakhia R, Rangarajan R, Wirth D. 2007. The role of DNA mismatch repair in generating genetic diversity and drug resistance in malaria parasites. *Mol Biochem Parasitol* 155:18–25. <https://doi.org/10.1016/j.molbiopara.2007.05.003>.
34. Gupta DK, Patra AT, Zhu L, Gupta AP, Bozdech Z. 2016. DNA damage regulation and its role in drug-related phenotypes in the malaria parasites. *Sci Rep* 6:23603. <https://doi.org/10.1038/srep23603>.
35. Aurrecochea C, Barreto A, Basenko EY, Brestelli J, Brunk BP, Cade S, Crouch K, Doherty R, Falke D, Fischer S, Gajria B, Harb OS, Heiges M, Hertz-Fowler C, Hu S, Iodice J, Kissinger JC, Lawrence C, Li W, Pinney DF, Pulman JA, Roos DS, Shanmugasundaram A, Silva-Franco F, Steinbiss S, Stoeckert CJ, Jr, Spruill D, Wang H, Warrenfeltz S, Zheng J. 2017. EuPathDB: the eukaryotic pathogen genomics database resource. *Nucleic Acids Res* 45:D581–D591. <https://doi.org/10.1093/nar/gkw1105>.
36. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
37. Galen SC, Borner J, Martinsen ES, Schaer J, Austin CC, West CJ, Perkins SL. 2018. The polyphyly of *Plasmodium*: comprehensive phylogenetic analyses of the malaria parasites (order Haemosporida) reveal widespread taxonomic conflict. *R Soc Open Sci* 5:171780. <https://doi.org/10.1098/rsos.171780>.
38. Bensch S, Canbäck B, DeBarry JD, Johansson T, Hellgren O, Kissinger JC, Palinauskas V, Videvall E, Valkiūnas G. 2016. The genome of *Haemoproreus tartakovskyi* and its relationship to human malaria parasites. *Genome Biol Evol* 8:1361–1373. <https://doi.org/10.1093/gbe/evw081>.
39. Galen SC, Borner J, Williamson JL, Witt CC, Perkins SL. 2019. Metatranscriptomics yields new genomic resources and sensitive detection of infections for diverse blood parasites. *Mol Ecol Resour* 20:14–28. <https://doi.org/10.1111/1755-0998.13091>.
40. Simons ND, Eick GN, Ruiz-Lopez MJ, Hyeroba D, Omeja PA, Weny G, Zheng H, Shankar A, Frost SDW, Jones JH, Chapman CA, Switzer WM, Goldberg TL, Sterner KN, Ting N. 2019. Genome-wide patterns of gene expression in a wild primate indicate species-specific mechanisms associated with tolerance to natural simian immunodeficiency virus infection. *Genome Biol Evol* 11:1630–1643. <https://doi.org/10.1093/gbe/evz099>.
41. Borner J, Burmester T. 2017. Parasite infection of public databases: a data mining approach to identify apicomplexan contaminations in animal genome and transcriptome assemblies. *BMC Genomics* 18:100. <https://doi.org/10.1186/s12864-017-3504-1>.