Primate malarias as a model for cross-species parasite transmission

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Abstract

Parasites regularly switch into new host species, representing a disease burden and conservation risk to the hosts. The distribution of these parasites also gives insight into characteristics of ecological networks and genetic mechanisms of host-parasite interactions. Some parasites are shared across many species, whereas others tend to be restricted to hosts from a single species. Understanding the mechanisms producing this distribution of host specificity can enable more effective interventions and potentially identify genetic targets for vaccines or therapies. With increasing ecological connections to local animal populations, the risk to human health is increasing. Which of these parasites will fizzle out and which have potential to become widespread in humans? We consider the case of primate malarias, caused by *Plasmodium* parasites, to understand the interacting ecological and evolutionary mechanisms that put humans at risk for disease. *Plasmodium* host switching from primates to humans led to ancient introductions of the most common malaria-causing agents in humans today, and new parasite switching is a growing threat, especially in Asia and South America. Based on a wild host-*Plasmodium* occurrence database, we highlight geographic areas of concern and potential areas to target further sampling. Finally, we discuss methodological developments that will facilitate clinical and field-based interventions to improve human health based on this eco-evolutionary perspective.

Introduction

Animals host an incredible diversity of parasites, here defined as organisms that live in or on another organism (the host) at some cost to the host, including microparasites (viruses, bacteria, fungi and protozoa) and macroparasites (helminths and arthropods). Science is only just starting to understand this diversity of parasites, with the vast majority of parasites yet to be documented (Dobson et al. 2008, Poulin et al. 2016, Carlson et al. 2019). Some parasites are highly host specific, meaning that they are found only on a single host species, while others are generalists that are able to infect multiple hosts. Hence, these symbiotic associations and their transmission represent a vast web of connections that can be mapped among host species (Poulin 2010; Gomez et al. 2013). These associations vary over time as parasites go extinct, speciate, and transmit across host species, with these processes influenced by evolutionary dynamics and geographic movements of the host species themselves (Combes 2001). Parasites also drive coevolutionary dynamics involving reciprocal selective pressures favoring host defenses and parasite adaptations to overcome those defenses.

The factors that drive the connections between hosts and parasites are central to major research programs in ecology and evolution. These associations, and changes to them, also impact human health. In particular, parasites and pathogens can shift to human populations (a zoonosis) and adapt to humans, in some cases evolving to become specialists on humans (Wolfe et al. 2007), as seen with HIV-AIDS, measles virus, and the malaria parasite *Plasmodium falciparum*. Given the massive and global extent of anthropogenic change and its impacts on disease-carrying hosts (Gibb et al. 2020), such events are likely to occur increasingly often. Cross-species transmission events are also important to animal health and conservation, with parasites having negative fitness consequences for animal hosts and contributing to extinctions (de Castro and Bolker 2005). Many of these negative outcomes results from cross-species transmissions from domesticated animals, invasive species, or humans (known as anthropozoonoses in the latter case). Finally, of course, the loss of a host causes

loss of parasites (i.e., coextinction, Koh et al. 2004, Dobson et al. 2008, Dunn et al. 2009). Given the important role of parasites in ecosystems, the loss of hosts can have cascading effects, with some authors proposing using naturally-occurring parasites as a marker of a healthy ecosystem (Hudson et al. 2006).

One of the most fundamental questions in disease ecology and evolution concerns the factors that shape the distribution of parasites across host species. A phenomenon of particular importance for global health is parasite sharing, which refers to the occurrence of a parasite in multiple host species. The distribution of parasites across hosts is influenced by three mechanisms. The first of these is co-speciation, with the diversification of the host resulting in diversification of the parasite. This scenario results in congruent host and parasite phylogenies, as found in primates and their pinworms (Hugot 1999). Co-speciation is expected to result in parasites specializing on particular hosts (or sets of closely related hosts). A second mechanism involves opportunistic transmissions from one host species to a new species, broadly known as a host shift. Once successfully infecting a new host, the parasite lineage may specialize on it. Finally, a generalist parasite may infect multiple hosts. The majority of parasites may fall into this category, with sharing either limited to a few hosts – as is the case of Ebola virus infecting bats, great apes and duikers – or to a wide range of hosts – as in the case of *Giardia* infecting many phylogenetically diverse species. In primates, for example, one study found that approximately 70% of known parasites are documented to infect more than one host (Pedersen et al. 2005).

A variety of methods have been used to investigate the distribution of parasites among hosts. One starting point is to produce a matrix of hosts and the parasites (Cooper et al. 2012), which is known as an incidence matrix in ecology. Another approach maps the occurrence of a parasite onto a phylogeny (Cooper et al. 2012), or compares host and parasite phylogenies, aiming to identify host shifts along with parasite duplications (i.e. within host speciation) and parasite extinctions (Charleston 1998, Huelsenbeck et al. 2000). Finally, a number of authors represent host-parasite incidence data as a bipartite network (Poulin 2010). As its name suggests, a bipartite network has two parts: one for hosts and another for parasites (Figure 1). Edges are placed between organisms in each part (but not within) based on the occurrence of a parasite in a host. One can then generate a unipartite projection of this bipartite network showing how hosts are connected through the parasites they share (or parasites are connected through the hosts that they share) (Gomez et al. 2013). A major risk in all of these approaches is that we rarely know all of the parasites in a collection of hosts, with some parasites or hosts studied better than others (Walther et al. 1995, Stephens et al. 2016). For example, terrestrial primate species are more likely to be sampled for parasites than arboreal primate species (Cooper and Nunn 2013, Poulin et al. 2016). Thus, a variety of approaches have been developed to deal with variation in sampling effort (Nunn et al. 2003, Teitelbaum et al. 2020).

Understanding the drivers of parasite sharing is especially relevant for public health and the potential transmission of parasites to humans. One major predictor of parasite sharing is phylogeny: more closely related species are expected to harbor the same parasite because they share underlying physiology, immune defenses, behavior, and in the case of intracellular parasites such as viruses or some protozoa, these hosts share similar cellular phenotypes, such as viral or parasite entry receptors. This is expected to influence patterns of generalism and opportunities for host shifts. The effect of phylogeny has been documented in primates, with research revealing that more closely related primate hosts have more similar parasite communities (using Jaccard's index as an ecological measure of community similarity) (Davies and Pedersen 2008b, Cooper et al. 2012). Similarly, a

study of rabies virus among North American bat species also found support for higher probability of host shifts among more closely related species of bats (Streicker et al. 2010), while an experimental study demonstrated an effect of phylogeny on viral titers for three viruses in *Drosophila* (Longdon et al. 2011).

In addition to phylogenetic relatedness, geographic overlap and phenotypic characteristics also predict parasite sharing. Human influences are also actively changing these processes, with likely consequences for human disease risk and primate conservation. Hosts that share parasites must overlap geographically with one another (or with some other host that acts as a reservoir to infect them both). The studies mentioned above found significant effects of geographic overlap, although phylogeny was a stronger predictor (Davies and Pedersen 2008a, Cooper et al. 2012). Similarly, across mammals, Albery et al. (2020) found that both phylogeny and geographic overlap predicted sharing of viruses. Phenotypes that lead to direct or indirect interactions between species, such as a shared diet, water source, or sleep site, will also facilitate parasite sharing. A variety of evidence supports these effects. In a study of primates, for example, Cooper et al. (2012) found that similarity in body mass predicted the similarity of parasite communities in pairs of species (together with effects of phylogeny and geography). In a study of bat viruses, Willoughby (2017) found that cave-roosting increases parasite sharing, along with geographic overlap and phylogenetic distance.

The range of hosts that a parasite infects can also be quantified using measures of phylogenetic host specificity (Poulin et al. 2011, Cooper et al. 2012). This concept is important in the context of human and animal health because it determines the potential for cross-species transmission, with phylogenetic host specialists generally only crossing narrow phylogenetic distances, while phylogenetic host generalists can cross a wider phylogenetic range of hosts. Thus, for emerging zoonoses in humans, we should be most concerned about phylogenetic host specialists arising from great apes and other primates, but also concerned about phylogenetic host generalists in other mammals. In one recent study, Park et al. (2018) used a database of >1400 parasite species and 404 mammal host species to quantify phylogenetic host specificity and its correlates. They found that that arthropods and bacteria are the most generalist, viruses and helminths are intermediate in generalism, and protozoa are the most specialist of the parasites in this database. Park et al. (2018) also found that close-contact transmission is most associated with specialization on fewer hosts. These analyses also revealed a pattern consistent with a "leaps and creeps" strategy by parasites, with some parasites mostly infecting closely related hosts, but occasionally taking a "leap" to less related hosts, where the parasite circulates again amongst close relatives.

Here, we consider host sharing in malaria parasites, with a focus on primates. Malaria parasites range from single-host specialists to wide generalists, with different malaria parasites infecting a broad range of animals, including birds, bats, primates, ungulates, and rodents (Galen et al. 2018). Host sharing of malaria parasites is driven by a mix of ecological and genetic factors, and our understanding of the process is biased by sampling of some hosts more than others (Faust & Dobson 2015). Among avian malaria species, previous studies revealed that malaria species are relatively generalist, infecting a wide range of host species, allowing them to spread easily in a new habitat (Gupta et al. 2019, Ewen et al. 2012, Galen et al. 2018), though the full diversity and ecology of these species is only starting to be appreciated.

We review parasite sharing between humans and our close primate relatives for a group of protozoan parasites that cause malaria from the paraphyletic genus *Plasmodium*, and discuss how evolutionary and ecological perspectives can inform the origin and virulence of emerging zoonoses

and pathways for vaccine or therapeutic targets. We focus on primate malarias because of their close evolutionary relationship to humans and known parasite sharing with humans that produces disease. Therefore, primate-*Plasmodium* relationships provide a unique and important system to demonstrate the links between ecological and evolutionary perspectives with direct medical relevance. Notably, known primate malarias include both generalists, such as malaria parasites *P. knowlesi* and *P. cynomolgi,* which infect Asian and *P. malariae/P. brasilianum*, which infects South American monkeys, as well as specialists, such as the malaria parasites commonly found in African great apes. The newly-identified diversity of malaria parasites in African apes, of the subgenus *Laverania—P. reichenowi, P. praefalciparum, P. billcollinsi, P. blacklocki, P. gaboni, and P. adleri*—tend to be host specific, though Ngoubangoye et al. (2016) demonstrate some permeability, including occasional evidence for human *P. falciparum* infections potentially in African ape and South American monkey populations. Figure 1 summarizes the infection relationships between the major clades of primate malaria parasites and the primates that they infect.

Database

To examine the ecological, evolutionary, and sampling processes that underlie the host-specificity of primate malaria parasites, we collated an occurrence database of published records of the location and species involved in wild primate infections. We also collated a database of NHP *Plasmodium* species occurring in humans. Evidence for malaria in lemurs is limited, with no whole genome sequences available, so we focus on Central/South American monkeys, Asian & African monkeys, and apes.

The primate malaria database. We build on the database published in Faust and Dobson (2015) and the Global Primate Parasite Database (Stephens et al. 2017). We combined the two databases and updated them with new publications from January 2015 to August 2020. Following the methods of Faust and Dobson (2015), we searched the terms "*Plasmodium"* followed by each "genus of primates" in PubMed and Web of Science between January 2015 and August 2020. For each publication, we recorded the name of the host species, the location where the samples were taken, the number of individuals sampled, the sampling method, the number of individuals infected, and the *Plasmodium* species found. We followed the phylogenetic naming used by Faust and Dobson (2015) for the database and for search terms.

The human zoonotic malaria database. We next built a database of nonhuman primate (NHP) *Plasmodium* species sampled from humans; that is, zoonotic malaria occurrences. We followed a similar approach, searching PubMed and Web of Science for studies published between January 2015 to August 2020 with the search terms "name of each *Plasmodium* species naturally found in primate populations" followed by "human." For each publication, we recorded the location of infection when indicated (or the location where the blood sample was taken if unavailable), the number of individuals infected, and the *Plasmodium* species. We focus on this time period because molecular methods have dramatically changed the taxonomy and identification of zoonotic malaria, and we aim to avoid misclassification from early studies. Additionally, given the recent rise in sampling, we expect that this time period captures the vast majority of zoonoses.

Figure 2 plots the primate and human presence of zoonotic malarias *P. simium*, *P. knowlesi*, and *P. cynamolgi*. Publications where samples were taken from primates from zoos, breeding farms, or otherwise with no known origins were excluded from the figures. To represent the forest cover, we used the Forest Cover Data from Hansen et al. (2013).

Zoonotic malaria is a major health burden in humans

Human-infecting malaria parasites are part of the paraphyletic genus *Plasmodium* (Galen et al. 2018; Sharp et al. 2020), though the evolutionary relationship between some primate malarias is unresolved due to a lack of genomic data. These protozoan parasites have an obligate *Anopheles* mosquito vector stage for sexual reproduction and transmission between hosts. Of the roughly 30 known primate malaria parasites, currently a handful are known to naturally infect humans regularly: *P. falciparum*, *P. vivax, P. malariae, P. ovale wallikeri,* and *P. ovale curtisi,* with growing evidence for *P. knowlesi*. Indeed, all human malaria parasites have a zoonotic origin from our primate relatives, ranging from ancient host switching of the two most common human malaria parasites, *P. falciparum* and *P. vivax*, to ongoing and emerging zoonoses including *P. knowlesi*, *P. simium,* and perhaps *P. brasilianum*. Figure 1 shows parasite occurrence by genus of primate and subgenus or clade of malaria parasite. Because of the lack of whole-genome data for some primate malarias, we focus on this higher phylogenetic level.

Ancient zoonoses maintained today by human-to-human transmission

P. falciparum and *P. vivax* are responsible for approximately 95% of all malaria infections in human populations today (WHO report 2017). Substantial progress in the last decade has filled out the *Plasmodium* phylogeny and informed the timing and host origin of these two species. Yet, large questions about parasite origins remain, particularly for *P. vivax*; the direction of host switching based on modern sample diversity can be unclear given a lack of model-based inference, and sampling is still limited for wild, often endangered, primates. Despite the zoonotic origin, today, these parasites are maintained by human-to-human transmission, probably with little input from original reservoirs and substantial evolution since the host switch occurred.

These two common human parasites are from distinct parts of the *Plasmodium* phylogeny and represent independent zoonoses; *P. vivax* is more closely related to rodent malarias, such as *P. berghei* and *P. chabaudi*, and *Hepatocystis* spp., than it is to *P. falciparum* and the other great ape malarias known as the *Laverania* subgenus (Galen et al. 2018; Sharp et al. 2020). Therefore, the historical naming of *Plasmodium* should be supported by more taxonomically consistent subgenus definitions based on the main clades (Figure 1).

Given the close phylogenetic relationship between *P. vivax* and multiple macaque malaria parasites, the primary hypothesis for many years was that *P. vivax* emerged in ancient human populations from macaques in southeastern Asia (Escalante et al. 2005; Neafsey et al. 2012). Consistent with an out of Southeast Asia serial founder effect, recent analyses of genome-wide variation in global isolates of human *P. vivax* show increasing linkage disequilibrium and decreasing diversity with distance from Asia (Daron et al. 2020). These patterns of genetic variation however may be difficult to disentangle from the confounding effects of modern distribution of *P. vivax* population sizes, with the largest burden in Asia, almost none in Africa (likely due to host genetic adaptations), and a recent introduction bottleneck in South America.

Recent findings of *P. vivax*-like parasite in wild African chimpanzee populations have questioned this long-standing hypothesis, proposing an African ape origin for *P. vivax* (Liu et al. 2010, 2014; Loy et al. 2017). Incomplete lineage sorting, and perhaps sampling biases, have made phylogenetic inference difficult, with support for *P. vivax* as both a sister group to ape *P. vivax* and for it as a subset of ape parasite radiation (Daron et al. 2020; Sharp et al. 2020). A high frequency of the Duffy-negative allele in Africa, which is highly protective against *P. vivax* infection, may support an African origin. However, interpreting host adaptations as evidence of parasite origin is complicated because the occurrence and distribution of adaptive variants are limited by multifaceted pressures

such as population size, available genetic variation, and random mutation. Models of the origin of Duffy-negative in sub-Saharan Africa suggest it rose in frequency only ~42,000 years ago (McManus et al. 2017), perhaps weakening the evidence for long-term co-evolution in the region. Both origin hypotheses remain plausible, and more geographic sampling, higher quality genomes, and clearer analytical inference will be needed to differentiate between these alternative scenarios.

P. falciparum was historically thought to be inherited from a common ancestor of humans and chimpanzees, which then co-evolved with their respective hosts into human *P. falciparum* and chimpanzee *P. reichenowi* (Escalante and Ayala 1995; Loy et al. 2017). Extensive sampling of great ape parasites using non-invasive fecal sampling has demonstrated the deep and previously unappreciated diversity of ape parasites, in the subgenus *Laverania*, the closest relatives to *P. falciparum*. The huge radiation of human *P. falciparum* seems to completely fall within the tree of the gorilla *P. praefalciparum*, interpreted as a recent ape-origin of the deadliest human malaria parasite from African apes, perhaps in the last ~10,000 years (Prugnolle et al. 2010; Liu et al. 2010; Sharp et al. 2020). This recent origin is also supported by the low levels of genetic diversity observed in global isolates of human *P. falciparum* compared to both other *Laverania* species and to human *P. vivax* isolates. Other *Laverania* species have not been found in human populations, even when geographically overlapping.

The other two parasites that historically and commonly cause malaria in humans, *P. malariae* and *P. ovale*, are less studied, causing fewer overall infections and less severe disease (Rutledge et al. 2017). *P. malariae* has close relatives in both African apes (*P. rodhaini*) and South American monkeys (*P. brasilianum*) (Collins & Jeffery 2007). The direction of host switching is not directly clear, and whole genome sequence data is rare (Sharp et al. 2020). However, the high diversity and presence of other divergent lineages related to *P. malariae* within African apes supports an ancient African origin followed by a recent switch from humans to South American monkeys, perhaps during the Transatlantic slave trade (Rayner 2015; Rutledge et al. 2017). Relatives of *P. malariae* seem to be able to easily infect a variety of primate hosts (Figure 1), and may represent a single species.

The human parasite *P. ovale* consists of two subspecies *P. ovale curtisi* and *P. ovale wallikeri,* and is mostly found in Malaysia and Africa (Duval et al. 2010; Rutledge et al. 2017). African apes harbor nearly identical *P. ovale curtisi*-like and *P. ovale wallikeri*-like populations*,* suggesting that the two human *P. ovale* species may have diverged in apes before their spread into human populations. However, these *P. ovale* parasites seem to occur very infrequently in apes in the wild, and limited genomic data is available making the direction and timing of host transfer unclear.

Ongoing zoonoses, with unknown human-to-human capability

In addition to the ancient zoonoses that founded modern human malaria species, multiple NHP malaria parasites are currently infecting humans, particularly in Southeast Asia and South America. Primate parasites related to *P. vivax* and *P. malariae* have close relatives that are shared regularly between NHPs and humans; at least for now, evidence of ape to human transmission of *Laverania* is still rare, but nonzero. Regular transmission of *P. knowlesi* from macaques to humans in Southeast Asia is the most common and well-established example of zoonosis, though several others have unknown or occasional spread, such as transmission of *P. simium* from NHPs, especially howler monkeys, to humans in South America.

Today, zoonotic *P. knowlesi* is the most frequent malaria-causing agent in Malaysia, and widespread in Southeast Asia from its macaque origin (Figure 2b) (Rajahram et al. 2019, WHO report 2014, WHO report 2019). Despite its high incidence, evidence for direct human to human (via a mosquito

vector) transmission has not been clearly demonstrated. Further epidemiological case tracing and genomic sequencing can confirm this possibility. Previously misdiagnosed as both *P. vivax* and *P. malariae*, the timing of *P. knowlesi* first shifting into humans is unclear (Singh et al. 2004). Though less common and well-understood, occasional natural infections of another macaque parasite, *P. cynomolgi*, have also been observed in Southeast Asia (Ta et al. 2014).

Figures 2b and 2c show the distribution of *P. knowlesi* and *P. cynomolgi* cases, respectively, in both humans and primates. Reported cases of *P. knowlesi* in humans have occurred throughout Southeast Asia, including Malaysia, Lao, Myanmar, Indonesia, Cambodia and Thailand, whereas primate infections have primarily been documented in Malaysia. Given primate and vector ranges throughout the region, the lack of *P. knowlesi* is more likely due to under sampling of primate parasites than their absence. *P. cynomolgi* is present in primates throughout South Asia, though the range often does not overlap with known human infections (Figure 2c) (Imwong et al. 2018, Grignard et al. 2019, Hartmeyer et al. 2019, Raja et al. 2020). Similar to *P. knowlesi*, this suggests variation in sampling efforts drives the estimated species distributions, especially outside Malaysia, which has undertaken large sampling efforts because of its high *P. knowlesi* burden.

South America is a new hotspot for emerging zoonotic malarias. Early experimental studies confirmed the possibility of human infection of NHP malaria parasites *P. brasilianum* and *P. simium* (Contacos et al. 1963, Deane et al. 1966). The first natural infections in humans were only described in 2015 and 2017, for *P. brasilianum* and *P. simium*, respectively (Lalremruata et al. 2015; Brasil et al. 2017). Because of their close morphological and genetic relationships to common human malaria parasites, previous zoonotic infections of *P. brasilianum* and *P. simium* have been classified as *P. malariae* and *P. vivax*, respectively. Further genetic and epidemiological studies are needed to clarify the extent of natural human infection and determine if their immediate origin is transmission from NHPs or from other humans. Indeed because of their great genetic, morphological, and immunological similarity, it is unclear that *P. brasilianum*/*P. malariae* and *P. simium*/*P. vivax* should be classified as different species, or as populations of two generalist species, *P. malariae* and *P. vivax*. Genomic data shows some support for differentiation between *P. simium* and *P. vivax* (Mourier et al. 2019), but is weaker for *P. brasilianum* and *P. malariae* comparisons.

We see in Figures 1 and 2a, *P. malariae*/*P. brasilianum* is found throughout South America and are able to infect a large number of primate species, whereas *P. simium* appears restricted to the Atlantic Forest region in southeastern Brazil, infecting only a few American NHPs. Currently, known outbreaks of primate *Plasmodium* in human populations are from limited locations (hatched colored regions); however, the presence of *P. malariae*/*P. brasilianum* throughout much of South America suggests further cases in humans are possible, and may already be occurring unreported since these parasites produce less severe disease. Currently most South American *P. malariae* cases are linked to international travel, but primates may serve as a reservoir for disease. Similarly, extra-Amazonian cases of *P. vivax* have been rising in Brazil, and further testing will be required to determine if some of these may be linked to zoonotic *P. simium* infections, misdiagnosed.

Other *Plasmodium* species naturally found in primate populations have been experimentally transmitted to humans, such as *P. inui* (Coatney et al. 1966), but have not been observed naturally. Such cases are worth monitoring for potential parasite genetic mutations or environmental changes that facilitate new zoonoses. *P. inui* is a strong candidate for future zoonotic transmission because it shares a host, some potential vectors, and environment with the known zoonotic parasites *P. cynomolgi* and *P. knowlesi* (Baird 2009, Coatney et al. 1966, Maeno et al. 2015).

Increasing threat of human malarias in primates (and eventually back to humans)
Malaria parasite sharing is not unidirectional; increasing human pressures on local primate populations are reintroducing human *Plasmodium* species into other primates, putting often endangered species at further health and conservation risk. Additionally, these parasites may later switch back into human populations, creating new human disease risk.

Because the great apes have close relatives of human-infecting *Plasmodium* circulating in their populations, it is hard to identify anthroponotic infections. However, the presence of drug-resistant mutations can be used to predict the direction of transfer as human to ape. Using this evidence, the human *P. falciparum* has been found recurrently in primate populations living near humans (Ngoubangoye et al. 2016, Prugnolle et al. 2013, Loy et al. 2017). *P. vivax*, *P. malariae*, and *P. ovale* have also been occasionally, but rarely, described in African apes (Kaiser et al. 2010; Duval et al. 2009; Hayakawa et al. 2009; Rayner et al. 2011; Sharp et al. 2020). Further whole-genome sequencing or typing of known diagnostic regions will be able to differentiate between these close parasite relatives and inform the risk level of anthroponoses.

South American malaria parasites demonstrate the risk to humans of anthroponoses. Now considered a zoonotic malaria parasite, *P. simium* is proposed to have originated as a human-to-howler monkey switch of *P. vivax* during European colonization and the Transatlantic slave trade in Brazil. An early hypothesis suggested that *P. vivax* come from a pre-Columbian introduction between 15 and 30 ka as humans first arrived. But new methodologies and a better sampling have supported a recent introduction, ~500 years ago with European colonization (Culleton et al. 2011, Van Dorp et al. 2020, Taylor et al. 2013, Hupalo et al. 2016, Rodrigues et al. 2018). Historical DNA from pre-eradication Spain supports a recent introduction and close relationship between historical Southern European *P. vivax* and modern South American *P. vivax* (Van Dorp et al. 2020). This is consistent with a host switch of historical *P. vivax* into howler monkeys to become what is today known as *P. simium*, supported by the low genetic diversity of *P. simium* and high similarity morphologically and genetically to *P. vivax* (Escalante et al. 1995, Lim et al. 2005, Mourier et al. 2019). Since then, *P. simium* has built up a handful of genetic differences from *P. vivax*, perhaps through drift or adaptations to a new host (Brasil et al. 2017, Mourier et al. 2019). Occasional cases of *P. falciparum* have also been reported in South American primates, though are rare and largely unconfirmed (Duarte et al. 2008; Yamasaki et al. 2011).

The history of *P. malariae/P. brasilianum* in the Americas is similarly complicated by sharing across multiple primate hosts. Today, the classification of these two parasite species seems to follow the host in which they are found—*P. malariae* for humans and *P. brasilianum* for NHPs—rather than parasite characteristics (Lalremruata et al. 2015). *P. malariae/brasilianum* is incredibly widespread in South American monkeys, and appears to circulate freely between primate and human populations and be a single anthropozoonotic species in South America. Similar to *P. simium*, the presence of *P. malariae/P. brasilianum* in American NHPs likely originated with a human to primate transmission associated with the Transatlantic slave trade, from African *P. malariae* (Collins & Jeffery 2007; Rutledge et al. 2017; Lalremruata et al. 2015). *P. malariae* is now rare in humans in South America, with most cases either introduced by international travel or potentially through new zoonoses from primate reservoirs.

Factors driving host sharing and specificity
In order to better predict future zoonoses or to build interventions for ongoing zoonoses, the drivers of NHP to human host switching has been a focus of empirical and mathematical modeling

studies. Controlled experimental studies have confirmed a variety of NHP malaria parasites can infect human hosts, and epidemiological and genetic studies have confirmed a small subset cause infection in natural settings. Yet, we are only beginning to understand the extent of zoonotic malaria cases, the rate of human-to-human transmission, and what ecological and evolutionary factors underlie the origin and spread of zoonotic malarias. Cross-species malaria transmission is a confluence of factors in the parasite, vector, and multi-host system.

Phylogenetic relatedness

Phylogeny is often a strong predictor of parasite sharing because of shared physiological, genetic, and environmental factors (further discussed in the Introduction). For primate malarias, the distribution of parasites across hosts appears more closely related to parasite phylogeny than primate phylogeny. Figure 1 summarizes parasite occurrence at the subgenus level by primate genus. *Laverania* species are primarily genus-specific, with *P. reichenowi*, *P. billcollinsi*, *P. billbrayi*, and *P. gaboni* present in chimpanzees, and *P. praefalciparum*, *P. blacklocki*, and *P. adleri* present in gorillas. In contrast, *P. malariae* (which we combine with *P. brasilianum* because they cannot consistently be differentiated morphologically or genetically) and *P. vivax* relatives often infect many NHP where they occur.

However, contrary to this pattern, *P. simium* and *P. vivax* have only been found in a handful of South American monkey genera despite extensive sampling, particularly howler monkeys, and *P. vivax* is rarely observed in African monkeys, though its further relative *P. gonderi* infects them. Consistent with the pattern of specialist *Laverania* and generalist *P. vivax*, human *P. falciparum* infects fewer NHPs than *P. vivax*, and fewer *Laverania* species seem to switch into humans today. The reason is unclear, however, because *Laverania* ancestral species switched to become the most widespread human-infecting malaria historically. That is, even rare host switching had led to pandemic malaria, whereas frequent host sharing of *P. vivax* relatives has had mixed ability to spread widely. Overall, the few numbers of subgenera make it hard to draw strong conclusions about the relationship between phylogeny and host specificity; *Laverania* is an outlier in both its specialism and the most divergent subgenus of parasites.

Ecology, environment, and behavior

An important ecological predictor of cross-species *Plasmodium* transmission is having shared vectors. This requires that the vector exhibits preferences for both host species and that the hosts overlap geographically. For example, the parasite with the highest disease burden in humans is spread by a group of *Anopheles* vectors, *Leucosphyrus*, known to bite both humans and other primates (though some species do show host preferences) (Galinsky and Barnwell 2009). *Anopheles cracens*, found mostly in peninsular Malaysia, is known to bite humans and NHPs, with some preference for humans. It is considered as one of the main vectors of *P. knowlesi* in human populations (Vythilingam et al. 2008, Jiram et al. 2012, Lau et al. 2016). The vectors of *P. knowlesi* include *A. balabacensis* and *A. latens*, found in Sabah and Sarawak, respectively. All of these vectors are commonly found in the forest or farms, implicating certain times and locations where transmission to humans is more likely to occur (Tan et al. 2008, Manin et al. 2016).

Multiple aspects of climate, including temperature and humidity, influence the lifecycle of mosquito vectors and their geographic range, and thus can influence parasite sharing. For each developmental stage of the mosquitoes—from eggs to adults—the temperature is one of the most important factors that influence the duration of the passage from one stage to another (Shapiro et al. 2017; Depinay et al. 2004; Mordecai et al. 2013; Mordecai et al. 2019). Because the parasite develops inside

the mosquito's salivary glands, the temperature will also play a role in parasite viability (Shapiro et al. 2017).

Anthrpogenic change such as deforestation, urbanization, demographic expansion, hunting is also believed to increase frequency of interaction with animal populations and therefore to induce a higher risk of spillover (Murray and Daszak, 2013; Wolfe et al., 2005; Loy et al. 2017; Plowright et al. 2017; Mwangi et al. 2016). These processes have been similarly linked to increased malaria transmission; however, urbanization also decreases malaria rates by removing mosquito habitats and increasing health infrastructure. For example, increasing demand for palm oil has reshaped land use patterns throughout Southeast Asia, increasing deforestation and potentially changing the distribution of vectors and hosts for *P. knowlesi* (Vijay et al. 2016). In Malaysia, deforestation and habitat loss has pushed macaque populations near to farms and semi-urban areas, where the vector *Leucosphyrus* occurs, leading to close contact between humans, primates, and vectors (Vythilingam et al. 2016, Sueur et al 2019). Forest loss can also lead to other environmental changes, such as an increase in local temperature or a modification of breeding sites, increasing malaria transmission (MacDonald and Mordecai, 2019; Yasuoka and Levins, 2007). The relationship between deforestation and malaria incidence is complicated and multidirectional because increases in human occupation of forested areas will initially increase exposure, though with time larger settlements often provide improved access to healthcare and urbanization. MacDonald & Mordecai (2019) found that deforestation increases malaria prevalence but that increasing malaria rates is associated with reduced forest clearing and economic activity in Brazil; this bidirectional relationship highlights the need for location-specific modeling to understand the drivers of parasite distributions and infection rates.

Ecological competition is also increasingly being appreciated as a driver of malaria parasite case distributions. As we make needed progress on *P. falciparum* elimination, the potential for zoonotic malaria to fill open niches must be monitored. Recent evidence suggests this tradeoff has occurred with elevated rates of *P. vivax* in regions with declining *P. falciparum*. A similar tradeoff has also been proposed for the rise of *P. knowlesi* (William et al. 2014; Cooper et al. 2020) as rates of *P. vivax* and *P. falciparum* declined. Monitoring open ecological niches will be important for preventing new emergences or resurgence of other malaria parasites.

Host-parasite genetic interactions

The genetic basis of host-parasite interactions for human malarias has revealed multiple pathways that mediate host susceptibility and co-evolution. Central to these is protein interactions during invasion of host red blood cells, as well as immune evasion. This raises the question, are the same genetic pathways important for between-species malaria transmission?

Host red blood cells have a variety of surface proteins that mediate interactions with parasites during parasite invasion of host red blood cells (Lim et al. 2017; Su et al. 2020; Ebel et al. 2017; Leffler et al. 2017; Kariuki and Williams 2020; Ebel et al. 2020; Band et al. 2021). For example, the Duffy antigen/chemokine receptor, *DARC* (also known as *ACKR1*), is a gene that encodes a surface glycoprotein. It is a classic example of adaptation in human evolution, with a single mutation producing the Duffy-null version of a surface glycoprotein that doesn't bind to *P. vivax*, preventing parasite invasion of host cells and most disease. Studies from multiple African populations have repeatedly found strong signatures of selection in human genomes at the *DARC* locus, likely because of its function preventing *P. vivax* malaria (McManus et al. 2017; Kwiatkowski 2005; Hamid et al. 2020; Pierron et al. 2018). This gene and its expression levels have also been implicated in

parasite invasion or disease in multiple other primates with *P. vivax* relatives, including South American and African monkeys (Costa et al. 2015; Tung et al. 2009; McHenry et al. 2010; Gunalan et al. 2019; Trujillo & Bergey 2020). For example, Tung et al. (2009) found a regulatory variant that impacts *DARC* expression in baboons, showing *in vitro* expression changes and differences in disease burden in a wild baboon population. Recently, Trujillo et al. (2020) found evidence for expression differences in *Colobus* monkeys correlated with parasite levels in individuals' blood. That is, gene families important for host-parasite interaction may be shared across primates, with important insights gained from differences in species-specific variation, including a role for regulatory variation.

Beyond *DARC*, transferrin receptor proteins are well-studied for their role in iron uptake by cells, but their role in parasite interactions is also increasingly being appreciated (Chan et al. 2020; Galinski et al. 1992; Gruszczyk et al. 2018). Another classic example of human adaptation involves resistance to *P. falciparum* malaria also through changes to red blood cell morphology, hemoglobin S (HbS), decreasing parasite invasion and in some cases causing sickle cell disease. More generally, Ebel et al (2017), found wide spread phylogenetic signatures of adaptation in hundreds of genes that interact with malaria parasites, suggesting a functional role. Together, these results suggest pathways known to be important for human-*Plasmodium* interactions are likely also important in NHP interactions with malaria. Yet, we know little about within or between species genetic or expression variation in these pathways across primates that may underlie host specificity and switching.

Parasites have complementary proteins that bind receptors on the surface of host red blood cells in order to enter host cells. Notably, Duffy binding proteins (DBPs) and reticulocyte binding proteins (RBPs) (Adams & Mueller 2017; Lim et al 2017; Su et al. 2020; VanBuskirk et al. 2004; Carlton at al. 2008). These are complements to the host binding proteins, such as those encoded by *DARC*. *in vitro* studies of *P. knowlesi* show that a junction does not form between host cell proteins and parasite when DBP is deleted (Singh et al. 2005). The DBP system in *P. vivax* shows incredible genetic diversity, including duplication events. However, *P. vivax* has recently been found to infect red blood cells of Duffy-negative people in Ethiopia and Madagascar, suggesting that alternative pathways to invasion exists (Ménard et al. 2010).

Recent work lead by Gruszczyk et al. (2018) explored alternative invasion pathways, identifying RBP complex binding to Transferrin Receptor 1 in hosts. Encoded by the *TFRC* gene, mutant cells with depleted Transferrin Receptor 1 expression show substantially less *P. vivax* invasion. Similarly, they showed that, in mice, antibodies against RBPs bound to the transferrin receptor in place of the parasite leading to a reduction in invasion (Gruszczyk et al. 2018; Chan et al. 2020; Galinski et al. 1992). Within-species genetic diversity across 11 genes in the *P. vivax* RBP family is likely to mediate parasite binding affinity, perhaps also suggesting a role for between-species variation in host interactions through these pathways. For example, different *P. vivax* isolates show different preference for binding to different location or type of host blood cells (Lim et al. 2016).

The importance of DBPs and RBPs in parasite invasion of host blood cells is a classic result. However, the role of this pathway in host specificity or host switching is largely unknown. Early genomic resources that derive from passage through monkeys are missing common RBP genes founds in human field isolates (Hester et al. 2013), suggesting potential for host specificity. Studies of targeted gene regions in *P. simium* found lower levels of diversity than *P. vivax* in multiple DBP genes (Costa et al. 2015). However, the cause was unclear: high drift from small population sizes during the transmission bottleneck, or positive selection removing diversity and increasing

differentiation while adapting to a new host? The first whole genomes from *P. simium* suggest positive selection based on higher differentiation (F_{ST}) between *P. simium-P. vivax* in DBP and RBP genes than genome-wide average, consistent with a role for DBPs and RBPs in parasite evolution to new hosts or host specificity (Mourier et al. 2019). However, the sample size is small, as in most primate malaria parasite studies because of the difficulty collecting samples, and the often low parasitemia levels of *P. vivax* relatives. Additionally, inference from summary statistic outliers can be difficult to interpret without understanding the population history and processes driving variation.

Without experimental studies to confirm function, caution is warranted. For example, population-genetic studies identified variants in RBPs that differed between human infections and those found in great apes; however, no significant difference in binding affinity was identified for parasites to ape versus human red blood cells, suggesting these RBP differences are not functionally critical for host specificity. This further suggests that there is a limited barrier for ape *P. vivax* to infect human populations, and they should be carefully monitored and treated.

Multiple genes in red blood cell invasion pathways have also been implicated in the host specificity of *Laverania* parasites, and host switching of *P. falciparum* based on comparative genomic approaches (Martin et al. 2005; Rich et al. 2009; Rayner et al. 2011; Otto et al. 2018; Galaway et al. 2019; Plenderleith et al. 2019; Proto et al. 2019). Galaway et al. (2019) used ancestral sequence reconstruction for reticulocyte-binding-like homologous protein 5 (RH5) and quantified differences in binding affinity for human, gorilla, and chimpanzee cells. They found that the inferred ancestral protein had similar binding affinity for gorillas and humans, perhaps aiding in early transfer of the population that became modern *P. falciparum* in humans. This is in contrast with the modern *P. falciparum* version of RH5, which is highly human-specific (Wanaguru et al. 2013). Changes in the protein binding systems for red blood cell invasion have also been implicated in *P. knowlesi* host switching (Moon et al. 2016; Dankwa et al. 2016).

In addition to red blood cell invasion, immune response is central to host-parasite interactions and co-evolution. These incredibly diverse genes, such as AMA1, MSP1, CSP, and the *var* and *pir* gene family variants, bind to host receptors, are involved in immune evasion, and have been associated with disease severity. *var* genes have been identified in ape *Laverania* species, but their variation and impact on disease progression outside of humans is unclear, particularly because the corresponding ape immune response and genetic variation at immune genes is difficult to study in natural populations. Understanding the strain-specific immune responses and range of natural genetic variation is particularly important because *var* genes such as PfEMP1 have been proposed as vaccine targets, and expression of *var* genes has been shown to change in response to treatments (Bachmann et al. 2019; Jensen et al. 2020). Su et al. (2020) further discuss the role of these gene families in host-parasite interaction, and further work expanding to non-human malarias will be important to understand the role of these genes in between-species susceptibility and host switching.

Discussion & next steps

Large-scale parasite sequencing and epidemiology has led to major insights into the evolution of drug resistance and transmission dynamics of human malaria parasites. We consider the steps necessary to bring these approaches to tackle emerging zoonotic infections, from predicting the next host switch to leveraging primate and parasite genetic diversity to design interventions.

Tackling the complexity of factors that influence parasite sharing and host switching

The distribution of malaria parasites across primate hosts is driven by a complex relationship of ecological and evolutionary factors. Understanding the primary mechanisms behind these parasite distributions will be important for predicting parasite emergence in human populations and designing effective interventions. Here, we considered three main processes underlying host specificity and distribution of parasites: parasite and primate phylogeny, environmental and behavioral processes, and hosts-parasite genetic interactions.

P. vivax and *P. falciparum* are in different subgenera in the paraphyletic *Plasmodium* group, and may have different genetic propensity and mechanisms for host switching. Broadly, *Laverania* species, including human *P. falciparum*-related species, seem more likely to be host specialists (but see Ngoubangoye et al. 2016). In contrast, *P. vivax*-related species, including *P. knowlesi*, *P. cynomolgi*, and *P. simium*, tend to be more amenable to infecting multiple primate hosts. Caution is warranted, and the robustness of this observation will depend on future sampling studies. Notably, while *Laverania* seem more specialized than other *Plasmodium* species, the human-adapted *P. falciparum* has been able to at least occasionally infect a wide variety of primates when ecologically aided by the most widespread and invasive primate on the planet—humans. Less is known about *P. ovale* and *P. malariae*, though they are more closely related to *P. vivax* than *P. falciparum*. Historically, *P. ovale* and *P. malariae* were thought to be sister clades, but with whole genomes available, it seems more likely that *P. ovale* is sister to *P. vivax* relatives (Sharp et al. 2020).

Though host specificity appears correlated with phylogenetic relationships, the difference in host specificity could derive from ecological causes instead of or in addition to phylogenetics. The phylogeny of *Plasmodium* largely corresponds to the primary geographic regions that the parasites are found—with *P. vivax* relatives are more common in Asia and South America, and *P. falciparum* and *Laverania* spp. in Africa—making phylogeny and geography difficult to disentangle causally. This geographic division between parasites may have differed historically given recent findings of *P. vivax*-like parasites in African apes. These findings support a hypothesis of African-origin of host switching *P. vivax* to humans. This hypothesis remains contentious, with the alternative of an Asian origin and the similarity in Africa based on more recent human to ape transmission. Further sampling and population genomic studies should aim to clarify the timing of these relationships. Faust & Dobson (2015) identified species and regions that are likely under sampled with respect to parasite diversity, suggesting new primate malaria parasites may occur in African monkeys and lemurs. Updating their database here, we see that even areas that are unlikely to introduce new parasite species, such as Southeast Asia and South America, still have substantial under sampling of known parasite diversity and range (Figure 2).

Host and parasite genetic factors play an important role in the ability of parasites to invade host cells and establish disease, or replicate and move through host systems to eventually be further transmitted. Therefore, host-parasite genetic mismatch may be a key potential barrier to host switching. However, the role of well-studied host-parasite interacting pathways, such as erythrocyte invasion or antibody evasion, in host specificity and the emergence of new human-infecting malaria species is largely unclear. This emphasizes the need for broader genetic sequencing of both NHP hosts and their malaria parasites to aid in prediction of human genetic susceptibility to potential emerging zoonoses. Experimental studies have identified key pathways in *P. falciparum* that likely played a role in host switching from its putative ancestors in gorillas (Proto et al. 2019; Galaway et al. 2019). *P. vivax*, however, is not amenable to long-term lab culture, so functional experiments have been limited and population-genetic data will play a central role (Luo et al. 2015; Udomsangpetch et al. 2008; Noulin et al. 2013).

Implications of genetic diversity for disease emergence and treatment

Beyond the direct clinical significance of rising zoonotic malaria cases in ongoing regions, downstream, this knowledge will be critical to predict and prevent primate malarias with the highest potential for spillover into humans and for human to human transmission (Barber et al. 2017; Brasil et al. 2017; Assefa et al. 2015; Sharp et al. 2020; Faust & Dobson 2015). Further sampling to understand host and parasite ranges and natural genetic variation, population-genetic studies of adaptation, and experimental work on the mechanisms of host specificity will inform whether natural parasite variation is likely able to bind with common host genes necessary for invasion and establishment of disease in other species.

Genes identified in host and parasites as important for host specificity and switching will also be potential pathways to target therapies and vaccines—both to prevent new malaria emergence in humans, and as an independent line of evidence of gene functional significance in disease. For example, DBPs are one of the proposed vaccine targets for *P. vivax*-related malarias because they induce protective immune responses in primate hosts (Costa et al. 2015; Chitnis & Sharma 2008; Mueller et al. 2015; Chen et al. 2016; Su et al. 2020). Host and parasite variants in the associate proteins that interact on red blood cells determines the immunity induced; therefore, an understanding of natural variation across primates will inform future vaccine design (Chen et al. 2016; Su et al. 2020). Indeed, the high levels of genetic variation in *P. vivax* have been proposed as a barrier to an effective vaccine (Lim et al. 2017).

Similarly, vaccines developed against *P. falciparum* have had mixed success based on the genetic diversity of parasites (Thera et al. 2011; Neafsey et al. 2015; Ouattara et al. 2015; Graves et al. 2016; Laurens et al. 2017; Bailey et al. 2020). Indeed, genes that interact with human antibody response are some of the most diverse in parasites (Tetteh et al. 2009; Amambua-Ngwa et al. 2012; Conway et al. 2015; Neafsey et al. 2021). Interestingly, the RH5 protein that has been implicated in host switching has been proposed as a strong vaccine target because of its low diversity and key role in host red blood cell invasion (Bustamante et al. 2013; Douglas et al. 2011)

The role of population genetic sampling and methods development

Within-country capacity building is particularly important for monitoring primate and zoonotic malaria. It is especially important to provide that capacity building for new genomic technologies are facilitating non-invasive sampling and field-based sequencing. Improving the ability of rapid diagnostic tests or designing capture methods to differentiate between closely related parasite species from different hosts—such as *P. vivax* versus *P. simium* or *P. falciparum* from other *Laverania* species—will be an important next step to stop human-NHP transmission pathways.

Recent studies have produced hundreds to thousands of *Plasmodium* genome sequences from around the world, particularly *P. falciparum* samples from Africa and Asia, and to a lesser extent *P. vivax* (Hupalo et al. 2016; Pearson et al. 2016; Amambua-Ngwa et al. 2019). An important next step will be exploring the phenotypic consequences and origins of the observed genetic variants hypothesized to play a role in host switching or transmission. To achieve this goal, functional tests are needed, together with population-genetic inference of adaptive versus neutral processes. Combing this information with temporally and geographically fine-scale data will allow us to link human activity to vector-borne disease prevalence, and aid in predicting and prioritizing emerging zoonoses. Connecting diverse information types will need to move beyond simple correlations to model-based

inference and a deeper understanding of the mechanisms behind human-environment-disease interactions.

Robust population-genetic methods to analyze the plethora of parasite samples are limited, however, leading many studies to directly apply summary statistics that are well-studied in humans to parasite data or interpret phylogenetic trees, with mixed applicability. This approach has proven useful in viral genetic epidemiology with high mutation rates and low or no recombination. However, interpretation is more difficult with incomplete lineage sorting, slower mutation rates, and other processes present in the eukaryotic malaria parasites. Over the past few years, newer work has developed identity-by-descent and linkage-based summaries of genetic variation in *Plasmodium* species (Schaffner et al. 2018; Taylor et al. 2019). These summary statistics capture information along a chromosome, rather than treating loci as independent. They provide information about recombination and are more suited to study recent population history. Applied to a variety of worldwide populations, these studies have identified potential hybridization, population structure, and relatedness (Oliveira et al. 2020; Neafsey et al. 2021).

A major challenge is that undefined expected neutral genetic diversity in malaria parasites limits population-genetic inference. *Plasmodium* parasites undergo a complex lifecycle through host and vector stages. Multiple steps involve processes expected to dramatically influence genetic variation, such as bottlenecks with transmission, rapid asexual population growth in primate hosts, and sexual recombination in vectors. The combination of these stages makes genetic variation difficult to predict or fit to common models, such as Wright Fisher (Parobek et al. 2016; Chang et al. 2015; Chang et al. 2013). That is, we lack a general understanding of the expected neutral patterns of genetic diversity in malaria parasites. In turn, common outlier approaches to detect loci of importance (e.g. for drug resistance or adaptation to new hosts), such as F_{ST} scans, are difficult to interpret and may produce spurious results.

A handful of studies have begun to address this challenge through simulation approaches, making substantial progress linking epidemiological and population-genetic models (Chang et al. 2015; Chang et al. 2013; Hendry et al. 2020; Watson et al. 2021). They represent an important first step, but progress is needed to incorporate realistic population and genome sizes in computationally tractable ways. These flexible simulation studies can be extended to other parasites beyond the current focus on *P. falciparum*. Beyond model building for an intuition of the population genetics of malaria infection dynamics, simulation-based studies also open the door for simulation-based statistical inference of complex processes (Beaumont et al. 2010). With this model-based inference, we can more confidently differentiate between transmission networks or characterize regions of parasite genomes with patterns outside those expected from demography alone.

As genomic data for a wider variety of parasites becomes available, inference from population-genetic methods will instead be limited by our ecological and biological understanding of the systems. For example, more accurate estimates of mutation rates, the complexities of generation times, recombination rates, mosquito birth and death rates, and co-infection probability will parameterize the models.

Conclusion: a view of interdisciplinary studies to move forward

Parasite transmission studies are often human-centric, focusing on animal to human transmission. We emphasize that between animal transmission also has direct medical and conservation impacts, and monitoring of primate diseases should be a priority. This is particularly timely given recent

conversations about long-term creation of reservoirs of SARS-CoV-2 into new animal populations, which could then serve as a new source for future evolution and spillover into humans.

A broader perspective on the role of humans in disturbing environments, changing climates, and changing species interaction patterns will work synergistically with genetic and epidemiological work. For example, studies have increasingly demonstrated the importance of climatic and anthropogenic change on parasite and vector life-cycles, but the ecology of the hosts is generally understudied. For example, mathematical modeling suggests group size plays an important role in disease transmission dynamics and infection rates (Patterson and Ruckstuhl 2013; Caillaud et al. 2013; Nunn et al. 2015), though these questions have generally not been explored for multi-host systems. Multi-host mathematical models can be a key tool in understanding the factors driving host sharing and infection distributions, especially with changes to local environments through climatic change and human influence. Better feedback or integration across disciplines, including mathematics, social sciences, and different fields of biology will be an important step, and builds on similar interdisciplinarity in other areas of evolutionary medicine.

Figures

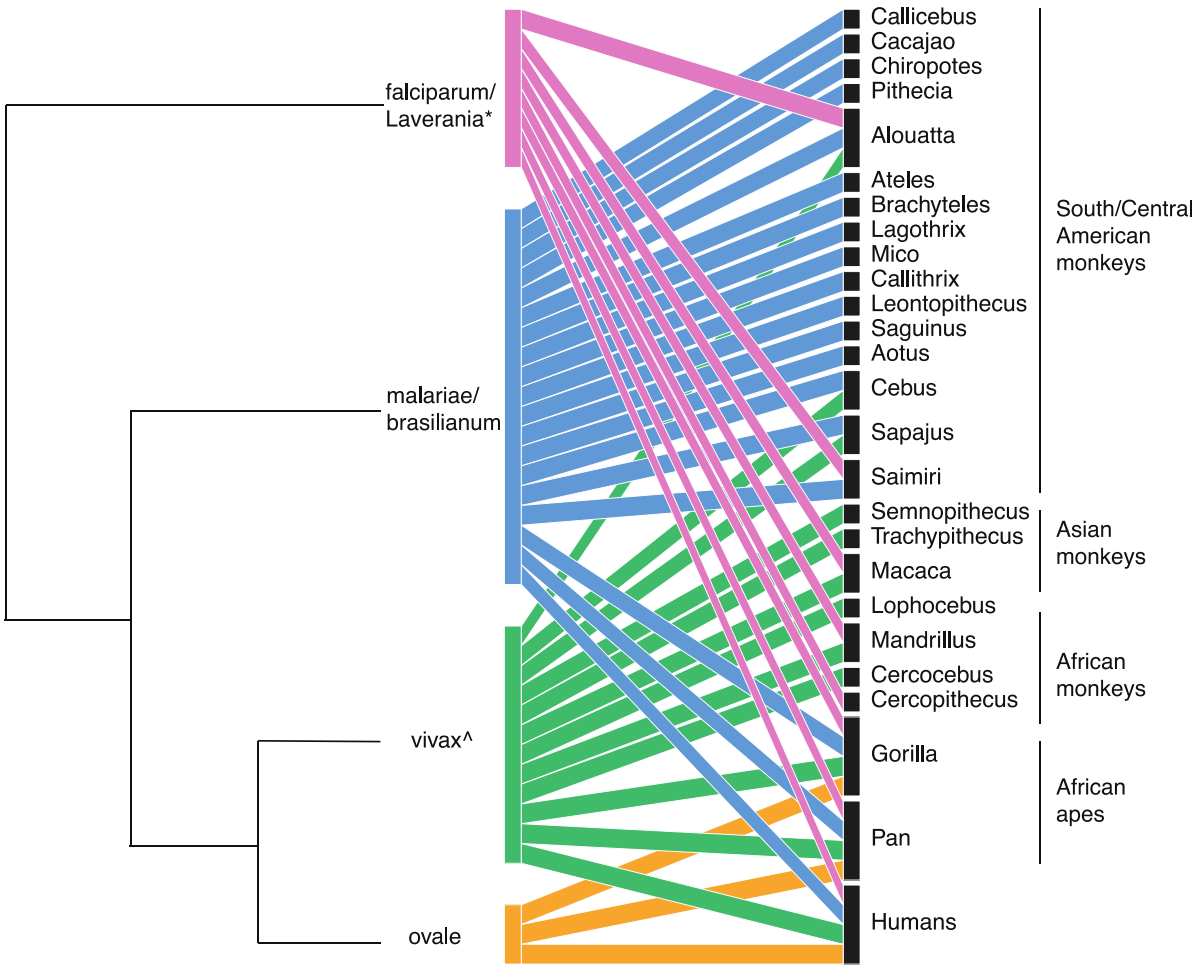

Figure 1: Bipartite plot of malaria parasite clade infection in primate genera. Phylogeny of malaria parasites follows Sharp et al. (2020), with parasites group by their clade (roughly corresponding to subgenus) based on the primary human-infecting parasite in that clade. Branch lengths are arbitrary. Colors correspond to the parasite clade. Figure made in R (Dormann et al. 2008).
**Laverania* clade includes *P. reichenowi, P. praefalciparum, P. billcollinsi, P. blacklocki, P. gaboni, P. adleri*
^vivax clade includes *P. simium, P. inui, P. cnomolgi, P. fragile, P. knowlesi, P. coatneyi, P. gonderi*

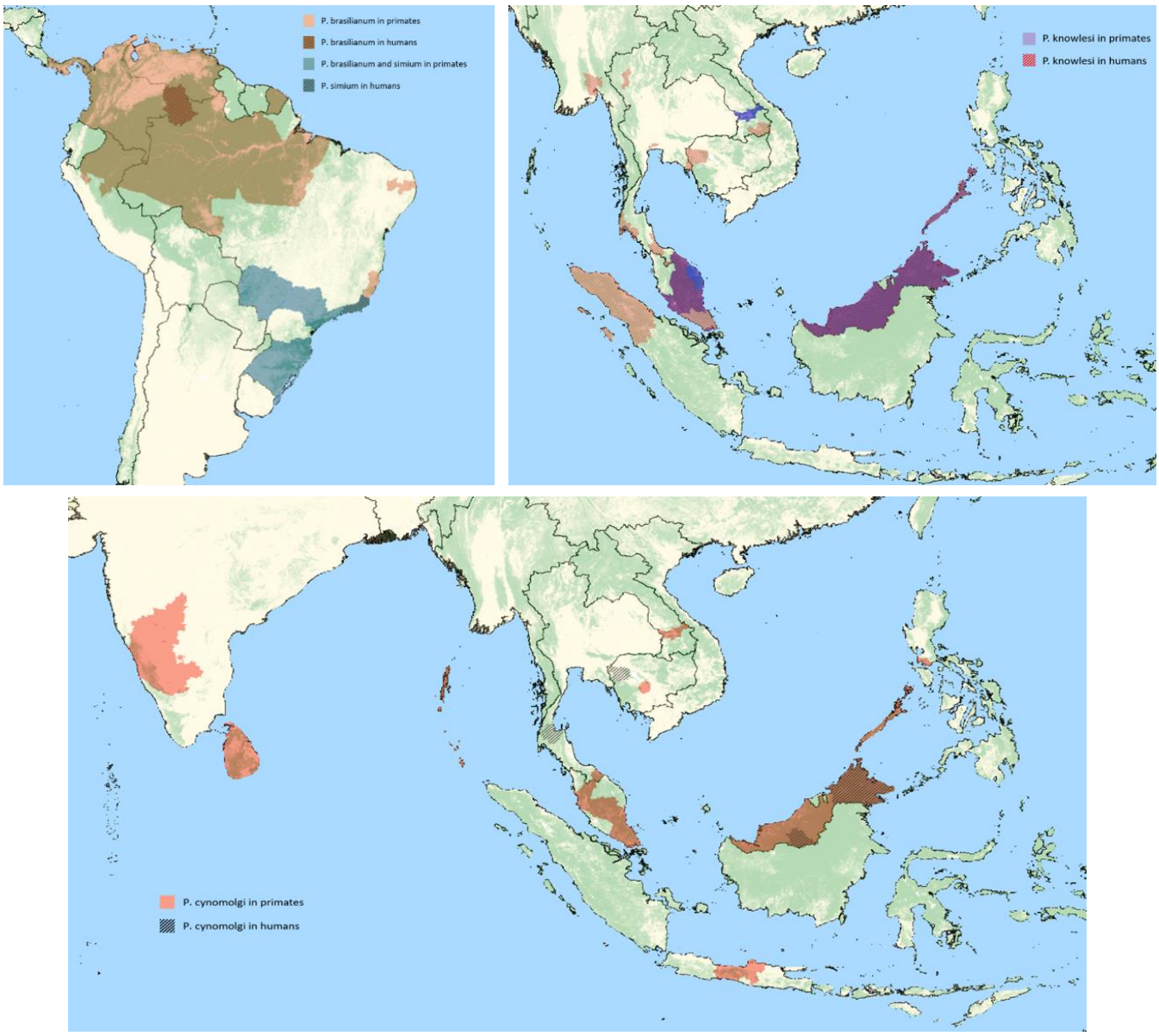

Figure 2: Geographic distribution of ongoing zoonotic malaria infections. Presence of (A) *P. brasilianum* and *P. simium* in South America, (B) *P. knowlesi* in Southeast Asia, and (C) *P. cynomolgi* in South and Southeast Asia. Colors represent cases in primates, and hatching represents cases in humans. Data available in Supplement, collation method described in Database.

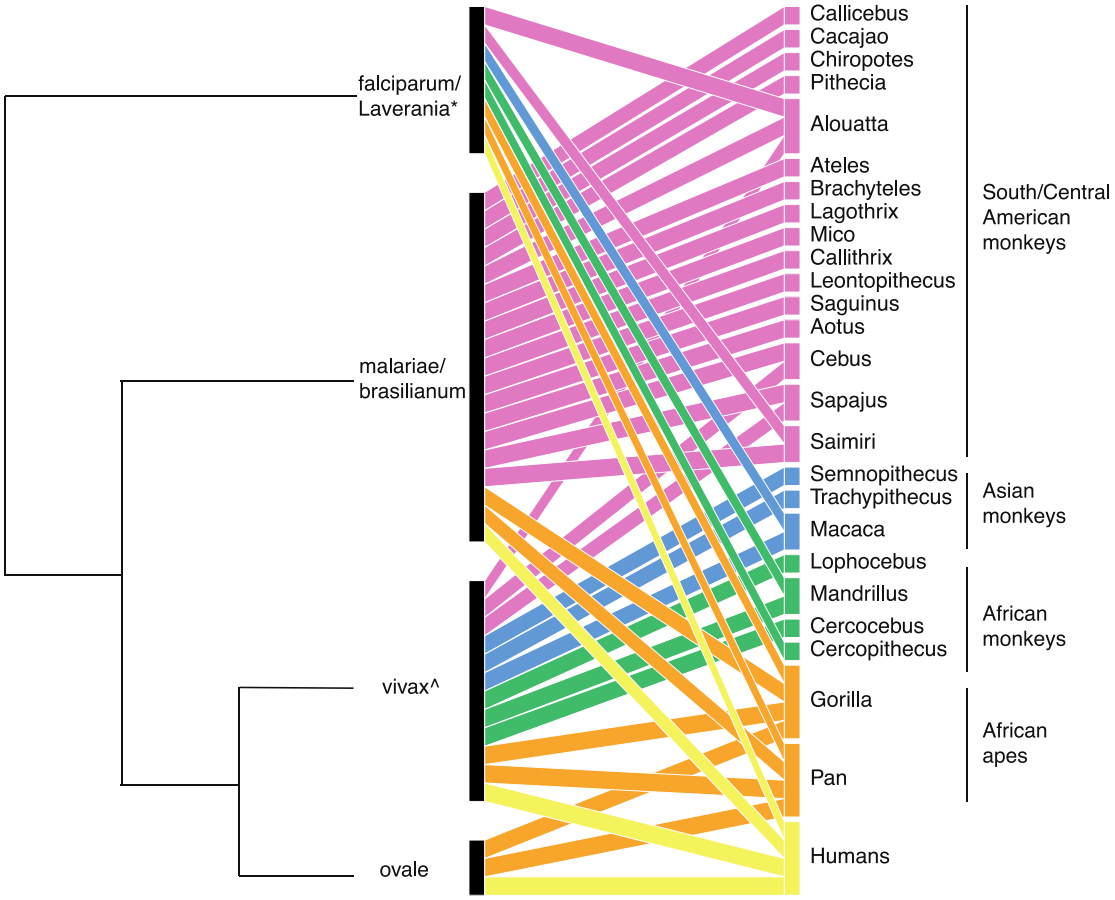

Figure 1—supplement 1: Bipartite plot of malaria parasite clade infection in primate genera colored by primate phylogeny. Phylogeny of malaria parasites follows Sharp et al. (2020), with parasites group by their clade (roughly corresponding to subgenus) based on the primary human-infecting parasite in that clade. Branch lengths are arbitrary. Colors correspond to the primate clade and phylogeny.
**Laverania* clade includes *P. reichenowi, P. praefalciparum, P. billcollinsi, P. blacklocki, P. gaboni, P. adleri*
^vivax clade includes *P. simium, P. inui, P. cynomolgi, P. fragile, P. knowlesi, P. coatneyi, P. gonderi*

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