Human migration, mosquitoes and the evolution of *Plasmodium falciparum*

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To date, coalescent analysis of the *Plasmodium falciparum* genome sequence has failed to provide a unifying theory regarding the parasite's evolution. While a better understanding of the evolution of the malaria genome will undoubtedly clarify the current controversy, the importance of the parasite's interplay with both the human host and mosquito vector cannot be underestimated. Changes in the population biology or ecology of either one of these species have consequences for malaria transmission and this was never more apparent than in the environmental changes brought about by the advent of agriculture.

Bioinformatic analysis of the malaria genome has allowed us to re-examine the evolutionary history of *Plasmodium falciparum*. Interpretation of this coalescent analysis must take into account that *P. falciparum* is merely one player in a three-way interaction, involving both the human host and the mosquito vector. Thus, information about the evolution of *P. falciparum* can also be gleaned from an examination of the history of these host species. The aim of this communication, therefore, is to examine the interaction of *P. falciparum* with its human host and the mosquito vector, and determine the extent to which their ecology and evolution has influenced the spread of malaria.

The age of extant Plasmodium falciparum

The closest relative of the human malaria parasite *P. falciparum* is the chimpanzee malaria parasite *Plasmodium reichenowi*. These species diverged ~5–7 million years ago, coincident with the divergence of humans and chimpanzees [1]. Phylogenetically, these two species cluster together and remain only distantly related to other human malarias and their simian relatives [1]. These features suggest that *P. falciparum* has been infecting our hominid ancestors for millions of years, and is indeed an ancient parasite; however, malariologists have long held differing views (see Refs [2,3]). Although the evolutionary trajectory of extant *P. falciparum* since its divergence from *P. reichenowi* is unknown, advances in molecular genetics have allowed researchers to investigate its age and evolution experimentally.

There are two different opinions concerning the evolution of extant *P. falciparum* generated by coalescent analysis of genome sequence data (reviewed by Refs [4–7]). The first asserts that the lack of synonymous polymorphism in some genes [8] and relative absence of single nucleotide polymorphisms (SNPs) in housekeeping gene introns [9] are indicative of a recent expansion from a single or limited number of progenitors, and that regions of diversity can be explained by positive selection [4,9]. The opposing view asserts that the parasite is ancient due to extensive polymorphism observed in some genes [10–12]. Thus, it is apparent that the genetic data proves contradictory and needs to be explained further. Some researchers have proposed that selective sweeps [7,12] are the most likely reason for the monomorphism observed in parts of the genome, while others think the most probable explanation is a parasite bottleneck [8,9]. An alternative explanation could rest in the unusually high AT content that is characteristic of *P. falciparum* [13], a feature that is suggested to have consequences for polymorphism accumulation [14,15].

The most recent common ancestor calculations from these two models of genetic evolution imply that either *P. falciparum* was present in our hunter–gatherer ancestors 100 000 or more years ago, with a relatively large effective population size that persisted [11]. Or, the parasite population went through a significant expansion around 6000 years ago from one or a few genomes [8,9]. This debate will continue until a better understanding of genome-wide SNP diversity is achieved.

Human evolution

Human genetic studies demonstrate that the frequencies of causative alleles for glucose-6-phosphate dehydrogenase (G6PD) deficiency and many red blood cell polymorphisms, such as sickle cell disease and α-thalassaemia, are highly correlated with the prevalence of malaria. Many of these malaria-protective polymorphisms have a negative phenotype associated with them, so to reach elevated frequencies they must be selectively advantageous. The high mortality associated with *P. falciparum* makes it a strong selective agent whereby the consequences of severe malaria outweigh the negative phenotype associated with the mutation. Ultimately, the origin of these protective polymorphisms must coincide with the presence of a selective agent, in this case *P. falciparum*, lest they be eliminated from the human population. This is not to imply that *P. falciparum* arose in tandem with these polymorphisms, but rather that it is at least as old. It is unclear whether *P. falciparum* underwent an expansion

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before the origin of these mutations or whether it already existed in a large population.

Sarah Tishkoff and colleagues investigated haplotypes containing two mutations associated with G6PD low activity alleles, which decrease the normal activity of the enzyme G6PD, and determined that these mutations have evolved independently within the past 3000–10 000 years [16]. Likewise, the malaria-protective human alleles that cause sickle cell disease and α-thalassaemia also appear to have origins within this timeframe [17,18].

Haplotype analysis has been carried out for a range of malaria-protective polymorphisms, and demonstrates that they vary according to geographical origin [18]. This means that different populations can be identified by the distinct haplotype that they carry. In addition, there is a patchy distribution of the protective polymorphisms throughout the malarious world, strongly suggesting that these adaptations arose in discrete areas after the racial divide of the indigenous human population [18–20]. These findings imply that *P. falciparum* has emerged and dispersed within the past 10 000 years and that escalating prevalence, rather than increased virulence of an already large population, resulted in the selection of malaria-protective polymorphisms. Thus, it appears that the human genetic data lend credence to a recent expansion of *P. falciparum*.

Modern humans and the development of agriculture

Plasmodium falciparum is dependent on its human host for survival and so examination of the parasite with respect to the location and behaviour of modern hominids is imperative to gain a better understanding of its history. Tracing the origins and dispersal of early hominids is a pursuit that evokes a deal of controversy and dissent, but evidence suggests that early forms of *Homo* originated in tropical Africa. Fossil records show that these early hominids diffused out and colonized much of Asia and Europe, and one of the genera subsequently gave rise to modern humans, *Homo sapiens*.

It is commonly thought that *Homo sapiens* arose in Africa ~100 000 years ago and subsequently migrated into the Middle East, southern Africa, Europe, central Asia, Pacific and finally into the New World (Fig. 1). There is considerable debate surrounding the number of migrations that occurred from Africa with recent evidence suggesting that it occurred in multiple waves [21]. Irrespective of how it happened, it is known that, by ~15 000 years ago, modern humans had colonized much of the earth's surface and were subsisting in small groups, employing hunter–gathering techniques to acquire food. Evidence suggests that they had a reasonably high standard of living, and successfully hunted and fished, as well as gathered enough plants, fruits and roots, to sustain small communities. Five thousand years later, this way of life changed dramatically with the dawn of modern agriculture.

The Middle East is generally considered the birthplace of agriculture, but it was not the sole site of development. There are believed to be three distinct regions where agriculture developed: the Middle East, which initiated the domestication of cereals; China, which developed rice farming; and Mexico, where pumpkins, beans and maize were first cultivated [22]. The development of subsistence farming was a crucial step in human history because it provided more control over food supplies and led to a vast increase in the potential number of people that the earth could support. The increased availability of food allowed populations to expand and led to the formation of large villages at first and, later, small cities. The ensuing population explosion resulted in migrations to new uncultivated land and, consequently, agriculture began to spread from its original focus. From the Middle East, agriculture spread in every direction into Africa, Europe and eastwards to India. Likewise, from the Chinese focus, there was a spread into Southeast Asia and eastwards to Japan.

As Fig. 1 shows, colonization of the Pacific region occurred at several different time points. The first wave of people arrived 40 000–60 000 years ago from Southeast Asia, settling in Australia, New Guinea, The Bismarck Archipelago and stretching as far east as the North Solomon Islands. The remainder of the Pacific was colonized in more modern times (from ~4000 years ago) by an influx of horticultural populations of Southeast Asian origins, who entered the western Pacific and moved along the coast in an eastern direction, finally reaching the far limits of Polynesia ~800 AD. Between both of these colonization events, there is not much evidence of movement around the Pacific region and into Southeast Asia. Agricultural populations are thought to have arisen independently in the highlands of Papua New Guinea (PNG), but contact with coastal populations is not believed to have occurred until after the secondary population migrations. Minimal contact between the Pacific region and Southeast Asia is interesting, in the context of malaria, because non-human primates failed to reach the Pacific and, consequently, there is no possible reservoir for malaria in this area. This suggests that the disease was introduced via population migrations from neighbouring areas in the secondary wave of migrations 4000 years ago (see Fig. 1).

The first evidence of a farming economy in Africa is in Egypt ~6000–7000 years ago and is believed to have derived from Asia Minor [23,24]. Technology and knowledge associated with farming quickly spread throughout Africa and the majority of populations changed their way of living accordingly. Two exceptions to this were Pygmy and Bushmen tribes, who, to this day, have retained a nomadic hunter–gather existence. The change in lifestyle from small nomadic groups to larger settled communities and the subsequent increase in population size would have, for the first time, provided conditions capable of sustaining *P. falciparum* transmission. Indeed, studies carried out by de Zulueta on Borneo demonstrated a significantly higher prevalence of parasites among farming populations than among hunter–gatherers, despite hunter–gatherers receiving substantially less medical care [25]. Although this study did not examine the genetic background of the two groups, and preclude an inherent susceptibility among the farming population, it does intimate that a difference in *P. falciparum* transmission exists according to lifestyle.

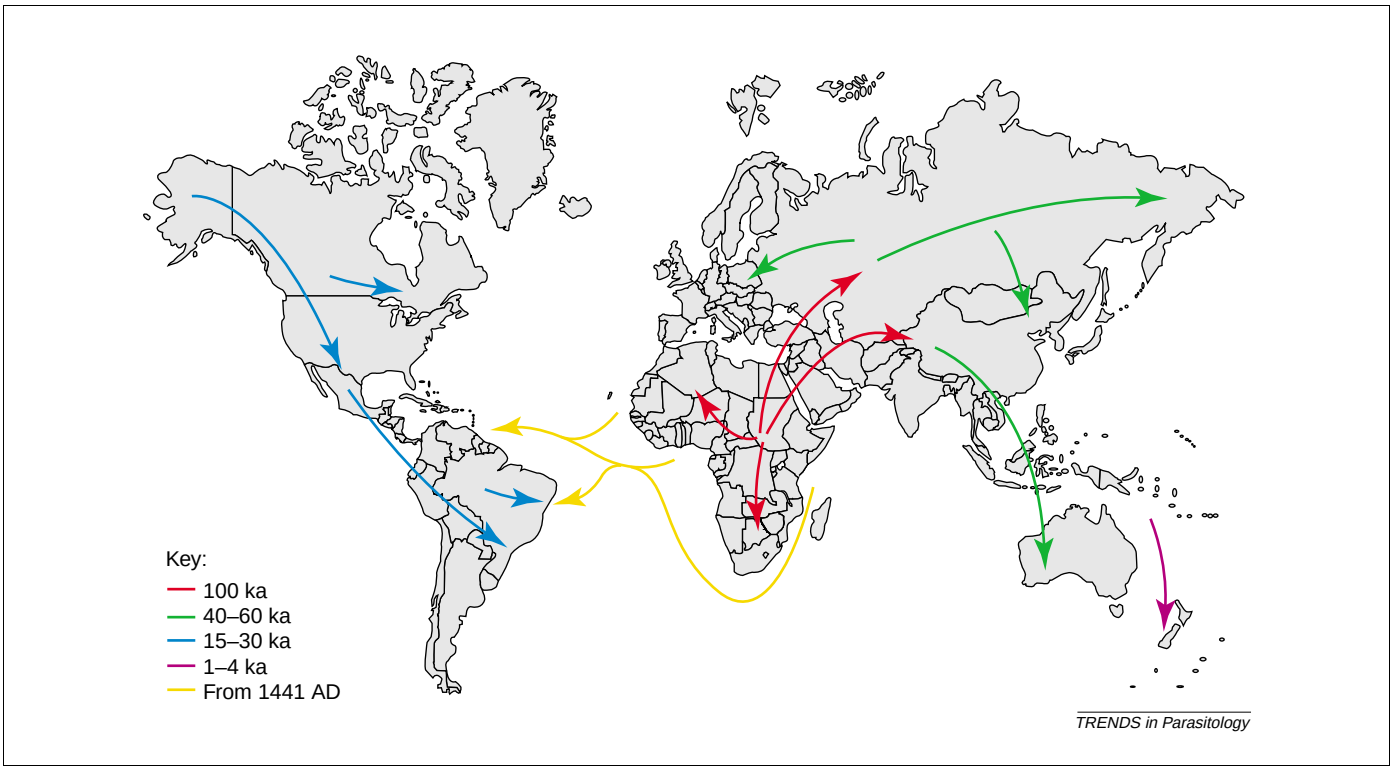

Fig. 1. The migration of modern humans from their point of origin, and movements resulting from the trans-Atlantic slave trade. Dates of movements arising from the trans-Atlantic slave trade were taken from Ref. [41]. Abbreviation: ka, thousands of years ago.

Indeed it is the opinion of Coluzzi [26] that *P. falciparum*, with its high pathogenicity and short-term survival in the host, would have faced a high risk of extinction in small, dispersed groups of hunters and gatherers. Consequently, Coluzzi favours a more recent origin of this parasite, probably dated within the past 10 000 years [26]. Because no evidence exists to the contrary, this assumption is based on: (1) the premise that *P. falciparum* did not infect a range of hosts such as other, now extinct, hominids; and (2) that it exhibited the virulence it does in the present day.

Malaria, mosquitoes and the dawn of agriculture

Following the agricultural revolution, the human population is estimated to have expanded from 5.3 million to 86.5 million during the 4000 years separating the Mesolithic period from the time of the earliest known village settlement at Jarmo in Iraq [6500 BCE (before the common era)] [27]. This would have had a phenomenal impact on population density and led to widespread population migration to avoid overcrowding. There was a need for extensive tracts of land to be converted to fields and, in many areas, this necessitated the destruction of forest habitats.

There is no evidence that the vector complexes implicated in the transmission of malaria in Africa – *Anopheles gambiae* and *Anopheles funestus* – have shifted from feeding on primates to feeding on humans [26,28]. This suggests that, initially, malaria transmission in Neolithic Africa was carried out by more zoophilic *Anopheles* spp. such as *Anopheles moucheti* and *Anopheles nili*. The evolution of more-anthropophilic taxa occurred later and, in the case of *An. gambiae*, was a direct consequence of the impact of humans on the surrounding environment [29].

Anopheles gambiae breeding sites are typically small, temporary, sunlit freshwater pools that occur naturally in savannah areas, but not in densely forested regions*. The advent of agriculture acted directly to increase the incidence of such breeding sites by destroying the thick vegetation cover and the absorbent layer of humus on the forest floor, leaving ideal conditions for water pools to develop [26]. Levels of rainfall were much higher in former rainforest areas when compared with savannah; hence, water pools persisted, providing continuous larval breeding sites. Before this forest destruction, *An. gambiae* would have been restricted to the forest fringes along low-level riverbanks. As the densities of *An. gambiae* and *An. funestus* increased, so did their interactions with humans, leading to several behavioural shifts, from exophily to endophily, and a switch from feeding on ungulates to feeding on humans [26]. Polytene chromosome analysis further substantiates this theory because the standard chromosome-two polytene arrangement has the highest frequency, nearly up to fixation, in afrotropical rainforests [30]. This chromosome arrangement is thought to be the most ancient, not only because of its primitive simplicity, but also because it is common to most members of the complex and could be a remnant of the original *An. gambiae* spp. before speciation [30].

P. falciparum evolution and anopheline diversity

Anopheles gambiae s.l. forms the most-efficient mosquito complex in terms of malaria transmissibility, boasting a high degree of anthropophily and endophily, as well as

* Gillies, M.T. and de Meillon, B. (1968) *The Anophelinae of Africa south of the Sahara: (Ethiopian zoogeographical region)*, South African Institute for Medical Research.

occupying a vast range of ecological niches. Its distribution in Africa is limited mainly to south of the Sahara with the north of Africa served by the relatively inefficient vectors *Anopheles pharoensis* and *Anopheles sergentii*. Figure 2 illustrates the main vector species currently implicated in malaria transmission, worldwide. *Plasmodium falciparum* first arose in tropical Africa [27,31,32]; therefore, prevailing mosquito vector populations would have heavily influenced its progression out of the continent, into Mesopotamia and beyond. The ancient civilization of Mesopotamia existed at a site of intersection for several different vector species such as those in Groups 4, 5, 6 and 8 in Fig. 2. Currently, the most efficient vector in this area is *An. stephensi,* but it is unlikely to have had any contact with the Sumerians or Babylonians because it was first detected by entomological studies in 1921, extending only a few hundred miles north of the Persian Gulf [25]. By the end of World War II, a survey showed that it had already reached Baghdad and, 20 years later, it was found near the Turkish border [25]. This recent expansion strongly suggests that it was a late introduction to the Persian Gulf, probably carried in the holds of ships sailing from Indian ports [33], and is substantiated by genetic studies demonstrating that the *An. stephensi* found in Iraq is the same as that in India [25].

In the absence of *An. stephensi*, malaria transmission in Mesopotamia was probably carried out by *Anopheles pulcherrimus* [25]. *Anopheles pulcherrimus* is an inefficient malaria vector, however, in all likelihood, it was present in densities similar to or higher than that which exists today and, consequently, would have successfully transmitted malaria by virtue of sheer numbers. Mesopotamia was a highly successful civilization, and historical writings make no mention of disease epidemics that could relate to malaria, so it appears to have largely been spared the ravages of severe disease. This correlates with the presence of an inefficient malaria vector and it is reasonable to suggest that the Middle East acted as a bottleneck to *P. falciparum* when it journeyed out of Africa.

It is important not to underestimate the impact of mosquito populations in the spread of *P. falciparum* and this can be seen in the case of the Indus civilization, as well as the introduction of the disease to Europe. Similar to Mesopotamia, the early Indus civilization was a highly successful society, but it came to an abrupt end in the middle of the second millennium BCE for unknown reasons. Evidence seems to suggest that human-imposed changes to the riverine forest environment led to an increase, and subsequent urbanization, of *An. stephensi*, and thus provided ideal conditions for endemic malaria to flourish with fatal consequences [33]. In Europe, climatic conditions are estimated to have stabilized to present day levels by ~4000 BCE [34], providing an environment suitable for *P. falciparum* transmission in much of southern Europe. Historical literature, however, provides no definitive proof of falciparum malaria in Europe until the beginning of the Modern era, which leaves a major question as to why such a time delay exists. The answer to this is unquestionably related to the refractoriness exhibited by the European vector species to tropical *P. falciparum*, as demonstrated by James in his malariotherapy experiments of 1932 [35].

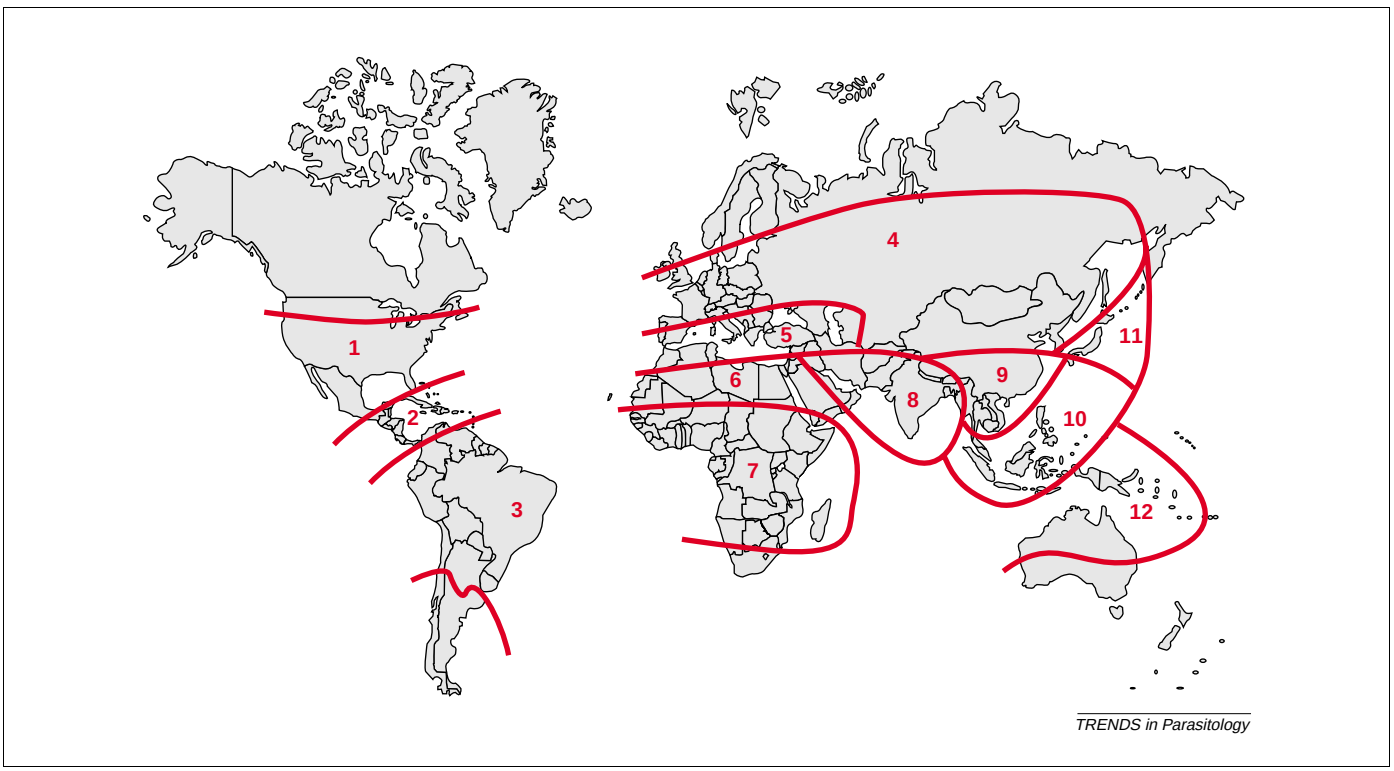

Fig. 2. The main malaria vectors by location. Key: 1, Anopheles freeborni and Anopheles quadrimaculatus; 2, Anopheles albimanus and Anopheles darlingi; 3, An. albimanus and An. darlingi (South America region); 4, Anopheles atroparvus; 5, An. atroparvus, Anopheles sacharovi, Anopheles labranchiae and Anopheles pulcherrimus; 6, Anopheles pharoensis and Anopheles sergentii; 7, Anopheles gambiae, Anopheles arabiensis and Anopheles funestus; 8, Anopheles stephensi, An. pulcherrimus and Anopheles culicifacies; 9, Anopheles dirus and Anopheles fluviatilis; 10, An. dirus and Anopheles balabacensis; 11, Anopheles sinensis and Anopheles anthropophagus; 12. Anopheles punctulatus. Compiled from Ref. [42], available at the Anopheles database on: http://konops.imbb.forth.gr/AnoDB/Species/malariaspecies.html.

Further work [36,37] confirmed these findings, but it was not until 1982 that the extent of this refractoriness was fully demonstrated [38]. Thus, the European vectors appear to have acted as a natural transmission barrier providing yet another bottleneck in the evolution of *P. falciparum*.

Microsatellite evolution of *P. falciparum*

A present day global analysis of *P. falciparum* microsatellite variation using parasite samples from Africa, Thailand, PNG and South America [31] provides tremendous insights into its population structure and evolution. The influence of human migration is clearly evidenced in the genetic relationship among global populations of *P. falciparum*, as visualized in Fig. 3.

Levels of diversity are highest in African populations, intermediate in PNG and Thailand, and lowest in South American sites. This is consistent with the hypothesis that *P. falciparum* originated in Africa and spread throughout the globe. Patterns of geographical differentiation substantiate this further. As Fig. 3 shows, parasite populations cluster according to their geographical location with the Thailand/PNG and African populations demonstrating strong clustering, which suggests that each of these form a discrete breeding population. By contrast, although the South American isolates cluster most closely to each other, they remain distinct. The Columbian isolates appear to form their own breeding population separate from the Brazilian and Bolivian isolates. This is no doubt a consequence of both the epidemic structure that *P. falciparum* typically exhibits in this area, compared to the endemicity demonstrated in Africa and much of Southeast Asia, but is indeed due to multiple, recent colonization events. The introduction of *P. falciparum* to South America is a relatively recent event, occurring within the past 500 years post-colonization by European populations and the introduction of the slave trade (Fig. 1).

The fact that the Thailand and PNG microsatellite haplotypes cluster together is very interesting. The best explanation is that this is a consequence of the introduction of parasites into the Pacific region from Southeast Asia by colonizing populations ~4000 years ago, rather than a more contemporary mixing resulting from the influx of Indonesian migrants into Irian Jaya [39]. This occurs despite the fact that both areas are served by different vector complexes: *Anopheles dirus* in Thailand and *Anopheles punctulatus* in PNG. This excludes the possibility of a vector-induced bottleneck, whereas the same cannot be said for the relationship between Thailand/PNG and Africa, where a variety of vector species inefficiently bridge the gap between *An. gambiae* (Africa) and *An. dirus* (Thailand).

The strong geographical population structure observed by Anderson *et al.* [31] implies that these parasite populations have been separated geographically or biologically. Modern day selective sweeps due to drug pressure have been shown to occur in *P. falciparum* [40] and it is suggested that similar sweeps could be responsible for the monomorphism observed [8,9,12]. However, if the monomorphism we see is due to selective sweeps, these sweeps must have occurred independently in different geographical locations. Although not impossible, this seems unlikely.

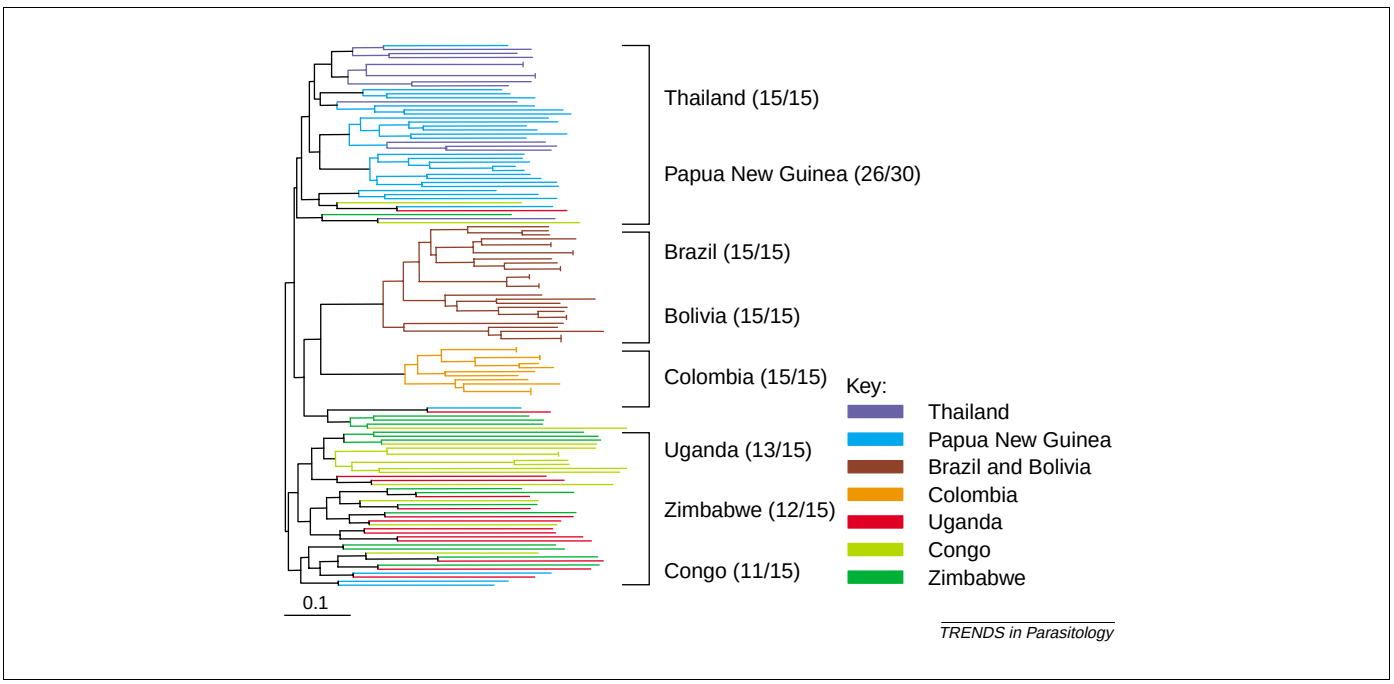

Fig. 3. Midpoint rooted neighbour-joining tree showing the relationships between *Plasmodium falciparum* haplotypes from eight different geographical locations. This tree is adapted from Anderson *et al.* [31]. Anderson *et al.* measured Nei's [43] genetic distance between parasite populations, and examined the genetic relationships among individual parasite haplotypes by counting the proportion of alleles shared between 12-locus haplotypes (*Ps*), and using the measure (1 − *Ps*) as a simple distance measure [44]. All trees were constructed using PHYLIP (Phylogeny inference package, Version 3.5, distributed by J. Felsenstein, 1993). Fifteen randomly chosen haplotypes from each location are included, whereas branches are coloured to show the origin of the parasites. Terminal branches of zero length mark identical haplotypes. The numbers in parentheses describe the proportion of parasites from a particular country that are found together in one cluster in the tree [31].

Conclusion

Human ecology suggests that *P. falciparum*, as we know it today, expanded ~6000 years ago, and subsequently spread throughout the world. Parasite genetic evidence both supports and refutes this claim. It seems clear that certain *P. falciparum* genes are indeed ancient, but that this antiquity does not extend throughout the genome as regions of monomorphism are observed. Whether this monomorphism implies a recent expansion from a limited number of progenitors or a series of selective sweeps remains to be established conclusively by further SNP analyses of the entire genome. However, it is remarkable that the parasite genetic evidence in favour of a recent origin coincides with one of the greatest changes in the evolution of modern humans. The impact of agricultural development on both human and mosquito populations was profound, and to dismiss it as an incidental development in the history of *P. falciparum* would be imprudent.

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