Vivax series:

Speculations on the origins of *Plasmodium vivax* malaria

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It is likely that *Plasmodium vivax* diverged ~2 million years ago from a group of malaria parasites which are now endemic in monkeys and apes in southern Asia. In those times, primates were spread throughout most of Eurasia and Africa, indicating an Old World location, but nothing more precise, for the place of divergence of *P. vivax*. From ~1 million years ago, the Ice Ages would have isolated human malaria, including *P. vivax*, into humid temperate or warm climate refuges around the Mediterranean, in sub-Saharan Africa and in south and east Asia. As there appears to be no record of humans in south and east Asia from 100 000 to 60 000 years ago, they might not have passed on their parasites, including *P. vivax*, to modern humans entering the region after this time. Today, all *P. vivax* might be descended from parasites which infected human populations in the Mediterranean region and in sub-Saharan Africa during the last Ice Age, between 100 000 and 20 000 years ago. Evidence for the latter is provided by the presence of very high frequency RBC Duffy negativity in sub-Saharan Africa.

Four species, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium falciparum* and *Plasmodium vivax*, are recognized as natural malaria parasites of humans. Outside tropical Africa, in Asia and the Western Pacific, and in Central and South America, the most prevalent of these parasites is *P. vivax* [1]. In sub-Saharan Africa, by contrast, cases of *P. vivax* are greatly outnumbered by those due to *P. falciparum* [1]. Indeed, in west and central Africa, where malaria transmission is otherwise more intense than anywhere else in the world, *P. vivax* is almost undetectable in the local human populations [2]. That it is present there at all is supported only by its occasional appearance in travelers returning from these regions [1,2].

Out of America?

How might the present distributions of the different species of human malaria parasite relate to their former distributions and to anything that might usefully be called their 'original' distributions? One possibility that has been discussed is that *P. vivax* might have been a parasite of New World monkeys that entered pre-Colombian human populations and was brought to the rest of the world in post-Columbian times [3]. The case draws, in part, upon evidence that a malaria parasite of South American monkeys, *Plasmodium simium*, appeared to be genetically [4,5], as well as morphologically [2], indistinguishable from *P. vivax*.

It has recently been found, however, based on polymorphisms of the 18S ribosomal RNA (rRNA) and the maternally inherited 35 kb plastid genome, that American and Asian/African *P. vivax* comprise genetically distinct populations [6]. Surprisingly, in phylogenetic analyses, *P. simium*, the *P. vivax*-like parasite of American monkeys, does not group with the *P. vivax* from humans in the Americas, but with the *P. vivax* populations of Africa and Asia [6]. The situation suggests that *P. vivax* does not, in fact, come from the Americas but was, on the contrary, introduced into the Americas from elsewhere; this would appear to have happened on at least two separate occasions [6]. One of these entries would probably have been from Asia bringing the parasites that are represented today by the *P. simium* population in New World monkeys. However, the *P. vivax* of present day American human populations appears to be of a different source.

On geographical and recent historical grounds, a likely candidate for that source is western Europe. Portugal, Spain, France, The Netherlands and England, were all countries in which *P. vivax* malaria was probably endemic by the time of their American colonial periods. In these circumstances, colonists from these countries would certainly have disseminated *P. vivax* to and from wherever in their travels there were competent *Anopheles* vectors, such as *Anopheles freeborni* in North America, *Anopheles albimanus* in Central America and *Anopheles darlingi* in South America. A molecular genetic analysis of material representing western European *P. vivax* could have been very revealing. Unfortunately, because autochthonous infections of *P. vivax* were eradicated from Europe by the last third of the 20th century, there is little prospect of retrieving such information unless from infected human remains.

In the Old World

As regards to malaria in Europe there is, however, a sufficient body of written observation and comment from antiquity onward, to provide a strong guide to much of the previous distributions of malaria in and around this

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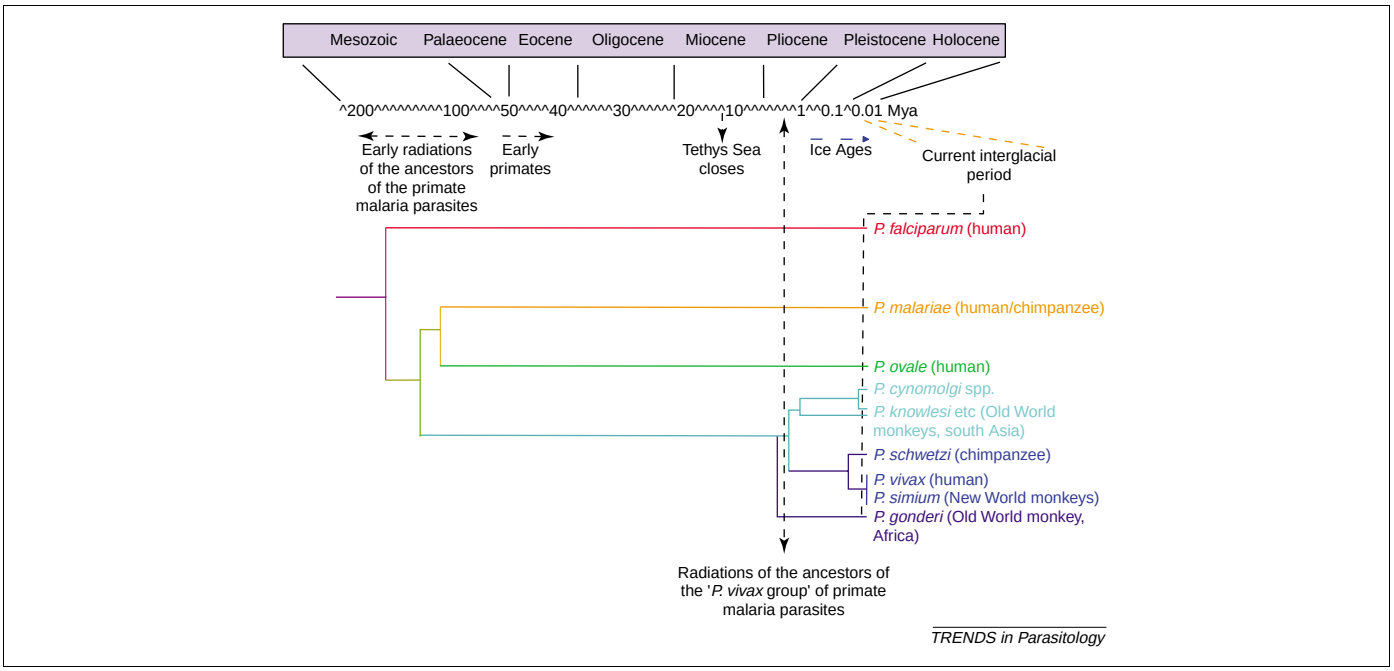


Fig. 1. Phylogeny of human malaria parasites and some related parasites of nonhuman primates, and time chart of events relating to their evolution. The information presented is compiled from Refs [2–5,13–17,19–23]. Abbreviation: Mya, million years ago.

region. One of the oldest, and the clearest, set of records is in the Hippocratic writings from Classical Greece [7]. These contain recognizable and ostensibly accurate descriptions of the environmental, seasonal and climatic circumstances associated with malaria in the eastern Mediterranean at ~450 BC Quartan and benign tertian fevers are described [7], as has been analyzed and discussed by experts such as Jones [8], Anderson [9], Zulueta [10] and Garnham [2]. They are unanimous in that these quartan and benign tertian fevers are almost certainly descriptions of *P. malariae* and *P. vivax* malaria, respectively. The presence of *P. falciparum*, malignant subtertian malaria, often fatal, is indicated in the views of Jones [8] and Anderson [9], as also appears reasonable from my own reading of the Hippocratic accounts [7]. This interpretation is questioned, however, by Zulueta [10]; Garnham [2] is silent upon the point.

The later presence of quartan and benign and malignant tertian malarias around the shores of the Mediterranean Sea is indicated by the observations of Classical and Roman writers [9]. Under these circumstances, and in view of the endemicity of malaria in Portugal, Spain and France well into the 20th century, it is hard to imagine that these south European colonial nations would not have suffered endemic malaria since long before the start of their modern colonial periods, especially given the evidence for quartan and benign tertian malarias (*P. malariae* and *P. vivax*) as far north at least as England by late Medieval/early modern times [11].

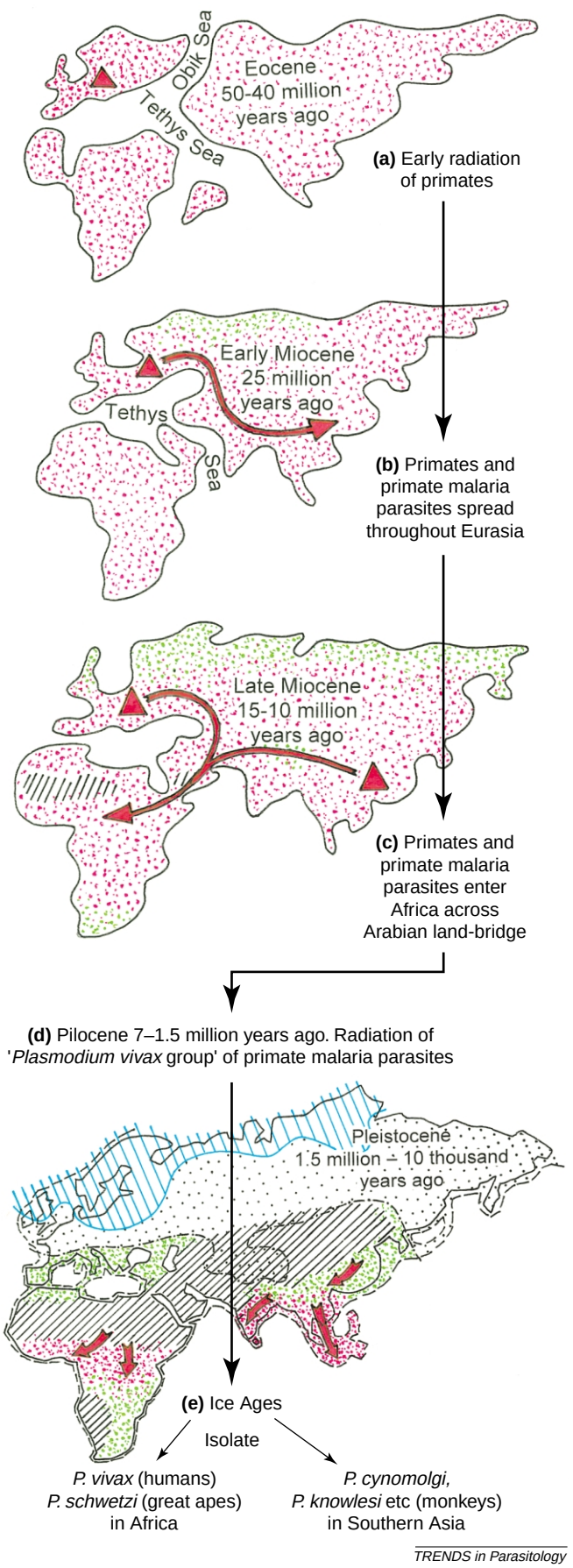
Based upon the presumed close phylogenetic relationship between *P. vivax* and several species of malaria parasites of monkeys and apes of southern Asia, it has long been argued that *P. vivax* originated in this region [12]. While the validity of these phylogenetic relationships is upheld by molecular analyses [3–5,13–17], consideration of the geological and climatic changes across the time period involved, does not necessarily lead to a hypothesis that *P. vivax* itself originated in southern Asian.

In the high and far-off times [18]

In the period from which the earliest primate fossils date, 60 million to 50 million years ago during the Paleocene and Eocene epochs [19,20], the major groups of the malaria parasites of primates had, according to molecular phylogenetic analyses [17], already long since diverged (Fig. 1). Primates were widely distributed in Europe and Asia [19,20] and, in the warm and humid climates of the times, primate malaria could likewise have been endemic across these continents. Meanwhile, the radiation of that group of primate malaria parasites which is represented today by *P. vivax* and *P. simium* of humans and New World monkeys, by *P. cynomolgi*, *P. knowlesi* and others in nonhuman primates of south Asia, and by *Plasmodium schwetzi* and *Plasmodium gonderi* in apes and monkeys of sub-Saharan Africa [2,16], was still in the far distant future (Fig. 1).

Throughout the Eocene and the Oligocene, Europe was isolated from the rest of Asia by a shallow seaway, the Obik Sea; an ocean passage, the Tethys Sea, separated both from Africa (Fig. 2a) [20,21]. It is unlikely, therefore, that either the primates or their parasites of Europe, Asia or Africa could have intermixed. However, by the beginning of the Miocene, ~25 million years ago, dry land had appeared between Europe and Asia (Fig. 2b), and from ~15 million years ago onwards, the Tethys Sea had closed, and Africa, Europe and Asia became a single landmass (Fig. 1, Fig. 2c). Complete exchange of the primate malaria parasites would, in principle, have become possible throughout the continents of the Old World.

Probably ~2 million to 3 million years ago [16], the *P. vivax* and *P. simium* line became separated from those that would lead to the malaria parasites of monkeys and



apes of southern Asia and the Western Pacific today [2] (Fig. 1). Now, primates including members of early human species were by ~2 million to 1.5 million years ago still dispersed widely across the continents of the Old World [19,20,22]. Therefore, no more precise location than somewhere upon the united African and Eurasian landmass can be identified with any confidence as the place where the *P. vivax* and *P. simium* line diverged or 'originated'.

Into the Icehouse

In the period shortly after these developments, the earth's climate began to change in a major way. For the past 100 million years at least, global climatic temperature has been falling slowly [23]. The cooling, however, has always been modulated by rhythmic changes in the earth's orbit and in other aspects of its orientation around the sun [22–24]. These produce exactly timed fluctuations in the amount of solar radiation approaching the earth's surface. Under these combined influences, at ~1 million years ago, the Pleistocene Ice Ages began [22–24]. For most of this time, each major glacial cycle has lasted close to 100 000 years including relatively brief, warm interglacial periods of 5000 to 20 000 years. The current interglacial approached present day climates and environments ~10 000 years ago [22,23].

During each Ice Age, ice sheets have expanded over large areas, especially of the northern hemisphere. In the most recent, which began ~120 000 years ago, and entered the warming interlude 18 000 years ago, the ice sheets spread from a center in Greenland, covering North America to south of the Great Lakes, Iceland, northern Britain and Ireland, Scandinavia, northern Russia and the north western rim of Siberia with ice to a depth of up to several kilometres (Fig. 2d). Permafrost extended into southern Europe, Central Asia, the Tibetan plateau, northern China and far eastern Siberia (Fig. 2d) [22]. The only warm and humid regions of the Old World continents were in sub-Saharan Africa and southern and southeastern Asia, while a cool to temperate humid region lay isolated in southern Europe and around the shores of the Mediterranean Sea (Fig. 2d) [22]. Separating these warm or temperate, humid refuges was arid land, much of it actual desert, extending from northern Africa through the Arabian peninsular and Iraq, around the shores of the Persian Gulf and the Arabian Sea to the plains of northwest India (Fig. 2d) [22].

With the exception of humans, and a single species of Macaque monkey, the Barbary Apes of northwest Africa, all other primate species retreated into one or other of the two warm climate refuges in sub-Saharan Africa or south and east Asia and the Western Pacific [19,20]. Human and nonhuman primate malaria would most probably have

Fig. 2. Changes in continental landmasses, climate and the distributions of primates and their malaria parasites in the Old World from the Eocene to the late Pleistocene. The information presented is compiled from Refs [2,19–24]. The associated movements of malaria parasites are conjectured as discussed in the text. Key: black cross-hatching, arid and desert regions; black dots, regions of permafrost; blue, ice sheets; green, regions of temperate climate; pink, regions of warm and humid climate; red triangles and arrows, general location and direction of change in the distributions of primates.

Box 1. Selection under *Plasmodium vivax* malaria for homozygous red blood cell Duffy negativity in human populations

At the start of the selection for any new mutation, its frequency in a population will be extremely rare. However, homozygotes for the mutation will be so rare as almost never to occur at all. Even if a homozygote should arise, the mutant genes will be returned to heterozygosity in the next generation with almost total certainty. Thus, when, as might be the case for the selection of red blood cell (RBC) Duffy negativity under *Plasmodium vivax* malaria, the force of selection applies only to the homozygous combination of the underlying mutation, then high frequencies of the gene might not be reached in a population for a 'very long time', or never.

This is, however, an extreme representation of the situation. Small population size and a tendency towards inbreeding would increase the probability of the homozygous state arising, once the mutation has entered the population in the first place. Moreover, a slight protective effect of the heterozygous state, as probably applies in the case of RBC Duffy negativity under selection by *P. vivax* [29], could be even more effective in speeding up the rate of selection.

It might be objected that, as an acute infection, *P. vivax* malaria is not, and might never have been, a very lethal parasite as, by contrast, *Plasmodium falciparum* can be. It does not follow, however, that infection with *P. vivax* malaria does not reduce the 'fitness' of the affected individual. Fitness, in this context, is the probability of leaving offspring. *Plasmodium vivax* malaria can certainly reduce the fecundity of individuals and human populations [28]. Depending upon the type of malaria endemicity, human population size and movement, the selective force of *P. vivax* malaria might be weaker or stronger [28].

In general, the rate of selection for high frequencies of homozygous RBC Duffy negativity under *P. vivax* malaria can be expected to be very slow compared with the rate at which the more powerful force of *P. falciparum* malaria selects for a condition such as the sickle cell trait in which the advantage to the host lies almost entirely, and strongly, in the heterozygous state. Selection towards equilibrium frequencies of the sickle cell trait under *P. falciparum* malaria, as has occurred in much of tropical Africa, is reckoned to have taken no more than 2000 to 3000 years [33]. The much slower rate of selection to near fixation of homozygous RBC Duffy negativity by *P. vivax* malaria in Africa is the key point upon which the human genetic argument for the long presence of *P. vivax* malaria in Africa, and for the generally much more recent arrival of *P. vivax* in locations outside Africa, is made.

been extinguished everywhere on the Old World continents except from these two refuges (Fig. 2d) and, in the case of humans, from around the Mediterranean shores.

Now, except in Africa, western Asia and the Mediterranean region, it appears that human populations elsewhere might have become extinct by ~100 000 years ago [25]. Moreover, there now appears to be a consensus that from this about time, possibly until ~60 000 years ago, all the ancestors of humans alive today were in Africa [25,26]. Here, they would have been totally isolated from the malaria parasites in other Ice Age refuges (Fig. 2d) [25,26]. If the south and east Asian human populations did become extinct before modern humans entered these regions from ~60 000 years ago, then so, too, would their parasites have died out, including *P. vivax*. This suggests that extant populations of *P. vivax* are likely to be of African or of west Asian/Mediterranean origin, where Neanderthal humans overlapped with the ancestors of modern Europeans until ~30 000 years ago [20,22,26].

A blood trail

There is independent evidence that *P. vivax* is an ancient endemic parasite of humans in Africa. It lies in a genetic imprint left upon modern African populations. In west and central Africa, 95% to >99% of indigenous populations are affected by a condition in which the Duffy antigen of the red blood cell (RBC) surface is totally absent [26]. Elsewhere across Africa, frequencies of RBC Duffy negativity range from 70% upwards and also in the adjacent Arabian peninsular can be as high as 50–60% [26]. In the rest of the world, however, RBC Duffy negativity is usually extremely rare, or it is absent [26,27].

RBC Duffy negativity is a homozygous condition that is due to a mutation in the promoter region of the gene controlling expression of the Duffy antigen on RBCs [27–29]. Carriers of this condition are totally refractory to infection with *P. vivax* malaria. Moreover, and in striking contrast to all other traits known, or believed, to protect against malaria – all of which have detrimental, and sometimes seriously detrimental, effects upon the carriers – the RBC Duffy negative condition carries no apparent cost. Therefore, a population exposed to *P. vivax* malaria might be expected, eventually, to become almost 100% homozygous RBC Duffy negative.

A recent analysis has produced an estimate that, within 95% confidence limits, selection for the Duffy negative trait began in Africa between ~97 000 and 6 000 years ago [30]. A likely period for the selection pressure to have begun must lie, therefore, in the region of 70 000 to 20 000 years ago. If *P. vivax* was the agent of selection for RBC Duffy negativity, then it must have been present in modern African human populations in the same time frame.

As has been discussed elsewhere [28,29] (see Box 1), selection for a trait, such as RBC Duffy negativity that is expressed only in the homozygous state, can be expected to take a 'very long time' - in the order of many thousands to tens of thousands of years. In populations to which *P. vivax* has been 'recently' introduced, therefore, (i.e. within less than the several tens of thousands of years expected to select for high levels of homozygous RBC Duffy negativity) RBC Duffy negativity should be very rare, or absent. This is, indeed, the case everywhere outside of Africa and the Arabian peninsular [26,27].

Historical evidence of *P. vivax* malaria in the Old World

On the above argument, *P. vivax* would be expected to have been present in modern human populations in Europe, Asia, the Western Pacific and the Americas, for less, or much less, than a few tens of thousands of years. During the present interglacial, *P. vivax* would have spread, probably at least 5000 years ago, from an African source across the Arabian peninsular into India and Central Asia and thence to the far east of Asia. Written accounts of diseases that suggest malaria, date from ~4700 years ago from China and 3500 years ago from India [31]. The epidemic, but not obviously lethal, nature of the diseases described from China, suggests *P. vivax* and possibly *P. malariae*,

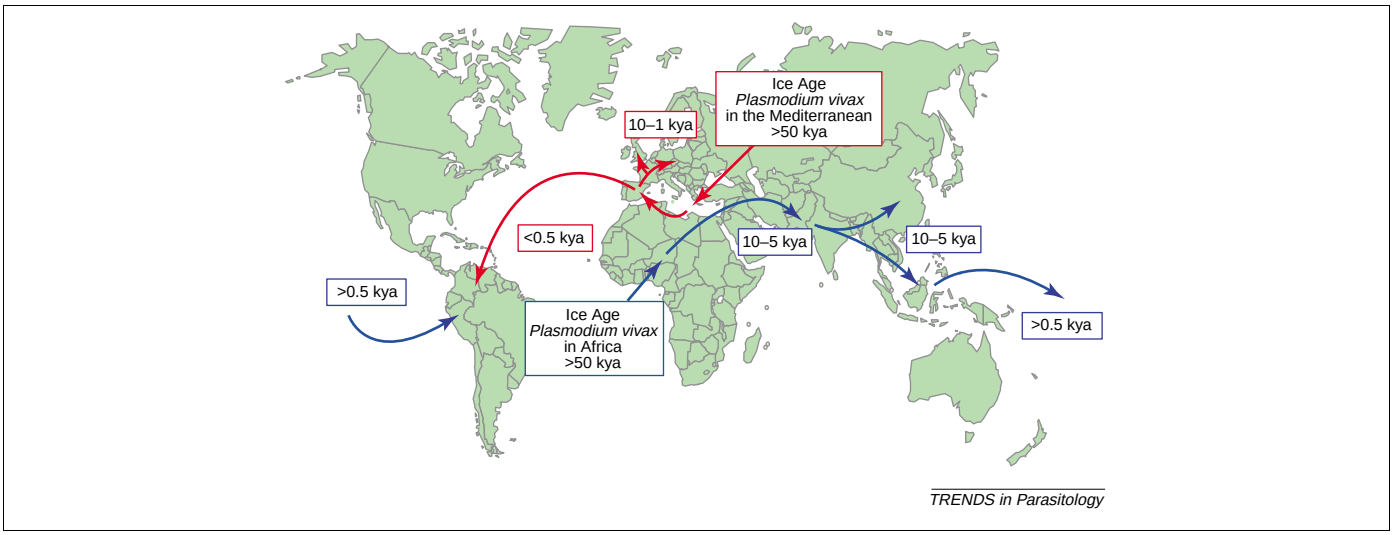


Fig. 3. Global dispersals of *Plasmodium vivax* malaria from the time of the Ice Ages. The blue arrows represent the postulated dispersals of a *Plasmodium vivax* stock isolated in human populations in sub-Saharan Africa until after the beginning of the present interglacial period, 18 000 years ago. Re-entering southern Asia, perhaps within the last 10 000 years, these parasites could have spread to the western Pacific region and thence, by fast sea transport, across the Pacific Ocean to the Americas in pre-Colombian times. The red arrows represent the dispersals of a stock of *P. vivax* that might have become trapped in the Mediterranean Ice Age refuge since before the beginning of the last Ice Age. Originally an infection of Neanderthal humans, this stock of *P. vivax* could have co-infected modern humans following their entry into the Mediterranean and southern Europe before the extinction of the Neanderthals ~30 000 years ago. It could then have survived in Europe into historical times, extended into northern Europe and finally have been transferred to the Americas by European colonists in post-Colombian times. Abbreviation: kya, thousand years ago.

rather than *P. falciparum*. All three species of malaria parasite appear to be represented, however, in Indian Vedic and Brahmanic writings from between ~3500 and 1900 years ago [31], and were probably present in the Mediterranean region by at least 2500 years ago [7–9].

Return to the New World

As already noted, the *P. vivax* of humans in the Americas appears to be genetically distinct from that of Asia and Africa [6]. If Europe, during its colonial period, was the source for the introduction of *P. vivax* into the Americas, it follows that European *P. vivax* must also have been genetically distinct from that of Asia and Africa. As indicated above, *P. vivax* could have persisted as a parasite of Neanderthal humans in the Mediterranean Ice Age refuge (Fig. 2d) isolated from other *P. vivax* stocks in sub-Saharan Africa and south and east Asia. From sometime after ~100 000 years ago, the Mediterranean *P. vivax* would have begun to infect modern humans entering this region. Following the extinction of Neanderthal humans, 30 000 years ago, the parasite would have continued to infect modern human populations into the present interglacial as the European *P. vivax* of recent times.

However, if Europe harbored such an ancient stock of *P. vivax*, why is the Duffy negative gene not as highly prevalent there as it is in Africa? The answer could lie in the circumstances of transmission of the parasites in each region (Box 1). In tropical Africa, malaria transmission could have been widespread, and human populations, however small or mobile, were frequently exposed to it. In the Mediterranean of the Ice Age, malaria transmission would almost certainly have been much more patchy. Small groups of hunter–gatherers might have had only rare and transient contact with malaria. Selection for RBC Duffy negativity, slow to get going under any circumstances, might have been a non-starter in southern European populations of the Ice Age.

And what of the apparent identity of *P. simium* of South American monkeys and African/Asian *P. vivax* of humans? An obvious explanation is that *P. vivax* was brought to the Americas by pre-Columbian human migrations from Asia. While this is unlikely to have been by the cold northern route across the Beringian land bridge from Asia to Alaska, it could have been achieved by ocean crossings from the Asian mainland or the western Pacific. As all the islands of the Pacific beyond Melanesia and Micronesia are *Anopheles*-free [32], the sea passage would have had to have been completed within the duration of a single *P. vivax* infection (i.e. probably within a year or two at most, allowing for relapse infections from hypnozoites).

If this is the explanation for the presence of an 'African/Asian *P. vivax*' in South America, why is this parasite not found there as a human infection today? Perhaps the original environments and lifestyles of native South American human populations were unable to sustain malaria transmission, or they might have learnt methods of environmental management that prevented its transmission. Thus, it might only have been after the arrival of Europeans and ensuing environmental disruptions, that *P. vivax*, in the form of the European import, became endemic among the human populations of the Americas. Meanwhile, the original Asian *P. vivax* had adapted to transmission in monkeys of South American forests as the parasite called *P. simium*.

A summary of the postulated movements of *Plasmodium vivax* over the period of the last Ice Age and current interglacial is presented in Fig. 3.

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