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Anopheles moucheti and Anopheles vinckei Are Candidate Vectors of Ape Plasmodium Parasites, Including Plasmodium praefalciparum in Gabon

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Abstract

During the last four years, knowledge about the diversity of Plasmodium species in African great apes has considerably increased. Several new species were described in chimpanzees and gorillas, and some species that were previously considered as strictly of human interest were found to be infecting African apes. The description in gorillas of P. praefalciparum, the closest relative of P. falciparum which is the main malignant agent of human malaria, definitively changed the way we understand the evolution and origin of P. falciparum. This parasite is now considered to have appeared recently, following a cross-species transfer from gorillas to humans. However, the Plasmodium vector mosquito species that have served as bridge between these two host species remain unknown. In order to identify the vectors that ensure ape Plasmodium transmission and evaluate the risk of transfer of these parasites to humans, we carried out a field study in Gabon to capture Anopheles in areas where wild and semi-wild ape populations live. We collected 1070 Anopheles females belonging to 15 species, among which An. carnevelei, An. moucheti and An. marshalli were the most common species. Using mtDNA-based PCR tools, we discovered that An. moucheti, a major human malaria vector in Central Africa, could also ensure the natural transmission of P. praefalciparum among great apes. We also showed that, together with An. vinckei, An. moucheti was infected with P. vivax-like parasites. An. moucheti constitutes, therefore, a major candidate for the transfer of Plasmodium parasites from apes to humans.


Introduction

Recent studies demonstrated that African great apes are infected by different Plasmodium species [1,2], among which four species that are traditionally regarded as human parasites (P. falciparum [3,4], P. vivax [5,6], P. malariae [5,6] and P. ovale [7]). These findings and in particular the discovery in gorillas of parasites that are genetically very close to P. falciparum (these parasites were named P. praefalciparum [2]), have changed paradigms concerning the origin of Plasmodium in humans and emphasized the risk of cross-species exchanges between apes and humans [1,2].

Plasmodium parasites are obligatory mosquito-borne pathogens and Anopheles mosquito might have played and still play a crucial role in their transfer to humans [8]. Inter-human transmission of Plasmodium species is well characterized in sub-Saharan Africa [9]. Conversely, nothing is known about the vector species that ensure Plasmodium transmission in African apes. To identify such vectors, authors [10] analyzed mosquito specimens collected close to nests of wild chimpanzees in Western Uganda, but failed to detect any infected mosquito. Yet, several species, including the Anopheles (An.) moucheti “sub-species” and species from the Anopheles nili group, might be candidate vectors [2]. Indeed, these species are major human malaria vectors, are present in and around forests in West and Central Africa and their distribution range largely overlaps with that of great apes. To identify the vectors of ape Plasmodium and evaluate the risk of transfers to humans, we started a field study in Gabon to capture Anopheles specimens in close proximity to wild and semi-wild ape populations. DNA amplification was used to detect Plasmodium-positive specimens.

Results and Discussion

Mosquito specimens were collected in the Park of La Lékédi (Bakoumba, Haut Ogooué Province) and the National Park of La Lobé (Ogooué Ivindo Province), in Gabon (Figure 1). Wild chimpanzees (Pan troglodytes troglodytes) and gorillas (Gorilla gorilla gorilla) live in these two wildlife natural reserves and they can be infected by ape Plasmodium (P. gaboni, P. reichenowi in Bakoumba...
and *P. reichenowi*, *P. GorB*, *P. praefalciparum* in La Lopé (Ollomo and Prugnolle, unpublished data). The Park of La Lékédi also hosts semi-wild chimpanzee and gorilla orphans in natural forest enclosures far from human dwellings. *Anopheles* specimens were captured using CDC light traps between October 2010 and April 2012 and identified by reference to standard morphological identification keys and by using molecular tools. Overall, 1070 female *Anopheles* specimens that belonged to at least 15 species were collected (Table 1). In the Park of La Lékédi, *An. moucheti moucheti* was the most prevalent species followed by *An. marshallii*, whereas in La Lopé, most of the specimens belonged to *An. carnea* (nili group).

Total DNA was extracted from all mosquito specimens and used as template to screen for the presence of *Plasmodium* DNA by PCR amplification of a portion of the parasite cytochrome b (cyt b) gene. This assay showed that eleven specimens from La Lékédi Park and four from La Lopé were infected by *Plasmodium* parasites. Sequence analyses revealed that three DNA samples from specimens collected in Bakoumba (BAK1 *Anopheles moucheti*, BAK2 *Anopheles moucheti* and BAK3 *Anopheles vinckei*) contained DNA that belonged to primate *Plasmodium* species (*P. vivax*-like in “BAK1 *An. moucheti*” and “BAK3 *An. vinckei*” and a *P. falciparum*-like parasite in “BAK2 *An. moucheti*”) (Figure 2 A), whereas the parasite DNA detected in all the other positive samples was genetically related to *Polychromophilus* sp. (bat Haemosporidia). Analysis of two diagnostic SNPs (at position 3575 and 3617 of the mitochondrial genome) that allow differentiating between human *P. falciparum* and gorilla *P. praefalciparum* [3] revealed that the parasite identified in the “BAK2 *An. moucheti*” isolate was related to *P. praefalciparum* (Figure 2 B). As the mosquito collection sites in Bakoumba were all located far from human dwellings (>10 km), it is very likely that apes or other non-human primates were the source of these parasites.

The BAK1 *An. moucheti* specimen infected by a *P. vivax*-like parasite was collected close to a semi-wild group of seven chimpanzees in the natural forest enclosure of La Miula (GPS position: S1°46’54.66’’; E12°58’42.53’’). It is likely that these chimpanzees constituted the source of parasite infection, although we cannot exclude infection after mosquito feeding on wild animals that are frequently observed near this site. The BAK3 *An. vinckei* specimen, also infected by a *P. vivax*-like parasite, was collected close to recent wild gorilla nests (GPS position: S1°44’33.24’’; E12°53’55.54’’). The detection of this parasite confirms that sylvatic transmission of *P. vivax*-like parasites occurs in Africa [3,5,6] and raises the possible role of apes as reservoir of *P. vivax* in West and Central Africa, as previously suggested [5,6]. Indeed, although in these regions 95%–99% of the population is resistant to *P. vivax* blood-stage infection because of the protective effect of Duffy-negative erythrocytes [12], *P. vivax* infections are frequently reported in travelers returning from these areas [13]. The existence of a sylvatic reservoir could explain these infections. Further analysis to compare apes and human *P. vivax* strains should be carried out to assess this hypothesis.

The BAK2 *An. moucheti* specimen (infected by *P. praefalciparum*) was trapped close to the sanctuary of three young gorillas (GPS position: S1°47’20.29’’; E12°59’35.20’’) that represent the most probable source of infection, although an infection from wild non-human primates cannot be excluded. *Plasmodium praefalciparum*, the closest known relative of *P. falciparum* in non-human primates, was first discovered in wild gorillas (hence the proposition that *P. falciparum* in humans is of gorilla origin [3]) and then also in a

![Figure 1. Location of the mosquito collection sites in Gabon.](https://example.com/figure1)

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Table 1. *Anopheles* mosquitoes collected in La Lékédi and La Lopé Parks between October 2010 and April 2012.

<table>
<thead>
<tr>
<th>Sites</th>
<th>La Lékédi</th>
<th>La Lopé</th>
<th>Both sites</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Collection dates</td>
<td></td>
<td></td>
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<td></td>
<td>Season</td>
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<td></td>
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<tr>
<td></td>
<td>24–27/04/2012 Rainy (long)</td>
<td>All collection dates</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25–30/03/2012 Rainy (long)</td>
</tr>
<tr>
<td><em>Anopheles</em> species</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>An. coustani</td>
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<td>–</td>
<td>1</td>
</tr>
<tr>
<td>An. eouzani</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>An. funestus</td>
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<td>–</td>
<td>15</td>
</tr>
<tr>
<td>An. gambiae ss M form</td>
<td>–</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>An. jebedensis</td>
<td>–</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>An. maculipalpis</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>An. marshalli</td>
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<td>15</td>
<td>194</td>
</tr>
<tr>
<td>An. moucheti moucheti</td>
<td>197**</td>
<td>3</td>
<td>69**</td>
</tr>
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<td></td>
</tr>
<tr>
<td>An. carnevatei</td>
<td>–</td>
<td>–</td>
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<tr>
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<tr>
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<td>An. vinckei</td>
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</tr>
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<tr>
<td>All species</td>
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<td>21</td>
<td>116</td>
</tr>
</tbody>
</table>

The table lists the number of females collected for each species. *An. sp*: when visual identification was not possible due to the deterioration of the morphological features.

*including one specimen infected with *P. praefalciparum.*

**including one specimen infected with *P. vivax*-like.

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Cercopithecus nictitans in Gabon [14], thus suggesting that monkeys could also be a source of infection. Our results suggest that An. vinckei and An. moucheti can support the sylvan transmission of P. vivax and P. praefalciparum in apes. Across all collection dates, the percentages of ape Plasmodium infected mosquitoes in Bakoumba were 0.74 and 3.22% respectively for An. moucheti and An. vinckei. As an indication, the sporozoite rates among potential vectors estimated in two Gabonese villages where the malaria transmission is endemic were 0.6 and 2.7% [15]. Nevertheless, considering the methodology we used (parasite detection done on whole mosquito with an unsuited molecular tool to discriminate parasite stages), these results are not sufficient to demonstrate the effective vector transmission. To definitively confirm this hypothesis it will be necessary to detect the presence of sporozoites (vertebrate infecting stage) in mosquito salivary glands by using microscopic, immunologic or molecular tools, and also to design additional studies to determine the trophic behavior of these mosquito species.

Anopheles moucheti, like mosquitoes of the nili group (An. nili ss and An. carnevalei), is present in the equatorial forests of Central Africa along river networks where their immature stages are usually found at the edge of rivers and streams [16]. An. moucheti is considered to be mainly anthropophilic in anthropogenized forest environments (i.e., villages) where it is a major human malaria vector.
vector [17]. Very little is known about its propensity to bite animals (and particularly non-human primates) because previous studies focused mainly on humans in rural and urban environments and never in sylvan environments where wildlife represents the main blood-source. Anopheles moucheti might actually be a zooanthropophilic vector that can readily operate Plasmodium transfers between apes and humans. Conversely, the role of sylvan Anopheles species, such as the strictly zooophilic An. vinckeii [10], is probably restricted to the transmission of Plasmodium among apes.

Conclusion

Here we identified two candidate vectors of P. vivax-like parasite and P. praefalciparum among apes in Africa. Our results suggest that An. moucheti, which is as a major Plasmodium vector in humans in Central Africa [17], might transmit Plasmodium in apes in the same geographic area and possibly also between apes and humans. Other Anopheles species, such as those from the nili group and An. marshalli that were previously implicated in human malaria transmission, were not infected by Plasmodium, but their density suggests a possible involvement. Further studies to gather entomological/parasitological and trophic behavior data are needed to solve the enigma of Plasmodium transmission in African apes and to evaluate the risk of transmission to humans at a time when contacts between humans and non-human primates are steadily increasing.

Materials and Methods

Collection of mosquito specimens

All mosquito collections were authorized by the Ministère de la Recherche Scientifique et du Développement Technologique du GABON (authorizations N° AR0009/10/MENERSI/CENAREST/CG/CST/CASAR and N° AR0006/12/MENERSI/CENAREST/CG/CST/CASAR). The "Agence Nationale des Parcs Naturels" (ANPN) and the "Centre National de la Recherche Scientifique et du Développement Technologique du GABON (authorizations N° AR0009/10/MENERSI/CENAREST/CG/CST/CASAR and N° AR0006/12/MENERSI/CENAREST/CG/CST/CASAR).

We consecutively collected mosquito specimens in two Gabon regions where great apes live and are known to be naturally infected by ape Plasmodium (P. gadi, P. reichenowi in Bakoumba [11] and P. reichenowi, P. GorB, P. praefalciparum in La Lopé (Ollomo and Prugnolle, unpublished data)). The landscape in Bakoumba and la Lopé corresponds to a forest-savannah mosaic. The climate of Gabon is equatorial with two rainy seasons that extend from February to April and from October to December. In Bakoumba (Haut Ogooué Province), mosquitoes were sampled in the private Park of La Lékédéi that houses wild and semi-wild (sanctuary for orphans) populations of chimpanzees (Pan troglodytes troglodytes) and gorillas (Gorilla gorilla gorilla) during four periods between November 2010 and April 2012. We also collected mosquito specimens in the National Park of La Lopé (Ogooué Ivindo Province) during two periods between October 2010 and March 2012. In this wildlife reserve, populations of both ape species are also present. Anopheles mosquitoes were captured using CDC light traps placed at ground level (1.5 m) in forest areas close to the ape sanctuaries (in the Park of La Lékédéi) and in several places where great apes were susceptible to rest (nests and feeding areas) (Table 2). All sites (including the sanctuaries) were located far from human dwellings (up to 10 km in the Park of La Lékédéi and up to 2 km in the Lopé Park) with scarce human activities between 8:00 AM and 5:00 PM.

Collected mosquitoes were morphologically identified by reference to standard morphological features [19], stored in liquid nitrogen, sent to the CIRMN and kept at −80°C until processed for molecular analyses.

DNA extraction, PCR amplification and sequencing

DNA was isolated and purified from the whole mosquito body using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer’s instructions.

DNA from mosquito specimens belonging to the An. gambiae, An. moucheti and An. nili complexes was used for species identification by using PCR-based diagnostic tools according to previously described procedures [20,21,22]. To search for the presence of Plasmodium DNA, total extracted DNA was used as a template for amplifying a 950 bp fragment of the parasite cytochrome b (cyt b) gene with a nested PCR protocol that was previously described in [11]. At the end of the second round of amplification, PCR-amplified products (5 μL) were run on 1.5% agarose gels in TBE buffer, and positive products were sequenced by Eurofins (Germany).

Phylogenetic analyses

We performed phylogenetic analyses using the obtained cyt b sequences and 27 previously published cyt b sequences from different Plasmodium species (see the supporting information file Table S1 for details concerning hosts and accession numbers). Multiple alignments of all partial cyt b sequences were carried out by using ClustalW (v 1.8.1 in BioEdit version 7.0.9.0) [23]. The maximum-likelihood (ML) tree construction was based on cyt b sequences of 917 nucleotides in length. The best-fitting ML model was identified according to the Akaike information criterion and by using ModelTest [24] and was a general time reversible model with gamma-distributed rates of variations (GTR +I+Γ) for the nucleotides. The highest-likelihood DNA trees and corresponding bootstrap support values were obtained with PhyML (freely available at http://www.phyml.org/).
available through the ATGC bioinformatics facility http://www.atgc-montpellier.fr, [25], by using nearest neighbor interchange (NNI) plus subtree pruning regrafting (SPR) branch swapping and 500 bootstrap replicates [26].

Supporting Information
Table S1 Parasite Cyt b sequences used in Figure 2. (DOC)

References

Acknowledgments
We are also very grateful to the "Agence Nationale des Parcs Naturels" (ANPN) and the "Centre National de la Recherche Scientifique et Technologique" (CENAREST) of Gabon that authorized this study and facilitated the access to the National Park of la Lopé.

Author Contributions
Conceived and designed the experiments: CP FP. Performed the experiments: CP BM BO NR PD JM EW FP. Analyzed the data: CP BM PD FP. Wrote the paper: CP FR DF FP.