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Recent studies have highlighted the large diversity of malaria parasites infecting African great apes (subgenus *Laverania*) and their strong host specificity. Although the existence of genetic incompatibilities preventing the cross-species transfer may explain host specificity, the existence of vectors with a high preference for a determined host represents another possibility. To test this hypothesis, we undertook a 15-mo-long longitudinal entomological survey in two forest regions of Gabon, where wild apes live, at different heights under the canopy. More than 2,400 anopheline mosquitoes belonging to 18 species were collected. Among them, only three species of *Anopheles* were found infected with ape *Plasmodium*: *Anopheles vinckei*, *Anopheles moucheti*, and *Anopheles marshallii*. Their role in transmission was confirmed by the detection of the parasites in their salivary glands. Among these species, *An. vinckei* showed significantly the highest prevalence of infection and was shown to be able to transmit parasites of both chimpanzees and gorillas. Transmission was also shown to be conditioned by seasonal factors and by the heights of capture under the canopy. Moreover, human landing catches of sylvan *Anopheles* demonstrated the propensity of these three vector species to feed on humans when available. Our results suggest therefore that the strong host specificity observed in the Laveranias is not linked to a specific association between the vertebrate host and the vector species and highlight the potential role of these vectors as bridge between apes and humans.

Plasmodium | *Laverania* | *Anopheles* | ape-to-human infection | African rainforest

Recent studies on great apes in Africa have revealed the existence of a large diversity of *Plasmodium* parasites infecting chimpanzees and gorillas, some being related to the most deadly human parasite *Plasmodium falciparum* (subgenus *Laverania*), others to the human parasites *Plasmodium malariae*, *Plasmodium ovale*, or *Plasmodium vivax* (subgenus *Plasmodium*) (1–4).

Within the subgenus *Laverania*, eight species are currently recognized. Among them, four species (*Plasmodium reichenowi*, *Plasmodium gaboni*, *Plasmodium billcollinsi*, *and Plasmodium billbrayi*) were observed only in chimpanzees and three (*Plasmodium praefalciparum*, *Plasmodium adleri*, and *Plasmodium blacklocki*) only in gorillas (2, 3, 5). In this subgenus, only *P. falciparum* infects humans. *In natura*, although these different host species cooccur in the same habitat where their ranges overlap, no transfer of *Laverania* parasites was ever documented between humans and apes or between gorillas and chimpanzees despite large sampling efforts (2, 3, 6). Similarly, ancient reciprocal transplant experiments of *Laverania* parasites between humans and apes (mostly chimpanzees) failed to produce infections (5). On the contrary, for parasites of the subgenus *Plasmodium*, like *P. vivax* or *P. malariae*, transfers were documented in natural populations (2, 4, 7) or during experimental infections (5). All this suggests therefore a strong host specificity of the *Laverania* parasites.

The origin of this host specificity in the *Laverania* could result from an incompatibility at the parasite/vertebrate host interface, at the vector/host interface, or at the parasite/vector interface (5). The first hypothesis has already received much attention and some studies have concluded to the potential existence of a genetic barrier precluding the transfer of parasites from one host species to another (especially from great apes to humans) (5, 8). This barrier would be the consequence of specific receptor/ligand interactions at the host red blood cell/parasite interface.

However, this hypothesis is at odds with observations made in conditions of artificial confinement such as in ape sanctuaries, where different host species (humans and great apes) live in close proximity and where human-to-ape transfers were documented. For instance, bonobos (*Pan paniscus*), from a sanctuary in the Democratic Republic of Congo, were found infected with *P. falciparum*, a parasite supposed to be human-specific (4). A similar phenomenon was observed in chimpanzees in a Cameroonian

Significance

African great apes were recently found to host a large diversity of parasites (subgenus *Laverania*) related to the main agent of human malaria (*Plasmodium falciparum*). Despite their close genetic relationships, these parasites are highly host-specific, infecting either chimpanzees or gorillas. This host specificity could result from incompatibilities between parasites and hosts or from a strong host tropism of the vectors. To test this second hypothesis, we performed a large entomological survey in the heart of the Gabonese rainforest (central Africa) to identify the vector species involved in ape *Plasmodium* transmission. Our results demonstrated that all ape parasites are transmitted by the same three vector species, thus rejecting the hypothesis that vectors could be responsible for the *Laverania* host specificity.

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See Commentary on page 5153.

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sanctuary (9), suggesting that host switches are possible under certain conditions, and hence that the species barrier might be porous. Therefore, other factors could contribute to this strong host/parasite association observed *in natura* in the Laveranias. One possibility could be that the segregation of the parasites in different hosts (i.e., chimpanzees and gorillas) is the result of specific interactions between parasites and mosquitoes (governed by genetic factors) or mosquitoes and hosts (i.e., feeding behavior).

The previous observations highlight the need to characterize the mode of transmission of the Laveranias among great apes and particularly to identify the mosquito species involved and their biology and dynamics in rainforests of Africa. To date, few studies have addressed this question, and the natural transmission of ape *Plasmodium* remains poorly understood, as does the transmission of other zoonotic *Plasmodium* (e.g., rodent *Plasmodium*). Indeed, most information about *Plasmodium* mosquito vectors were acquired in human contexts, concerned anthropophagic species (10), and are de facto difficult to transpose to natural rainforest contexts. Nevertheless, several authors have speculated that some of the main human vector species in forested areas of west and central Africa could be also involved in ape *Plasmodium* transmission (5). This is particularly the case of *Anopheles moucheti* and species from the *Anopheles nili* group that have overlapping habitats with great apes (11, 12).

We are aware of only two specific entomological studies aiming to identify vectors of ape *Plasmodium* undertaken to date. The first was carried out in western Uganda near nests of wild chimpanzees but failed to detect the presence of *Plasmodium* parasites among collected *Anopheles* species (13). The second survey, carried out in Gabon in areas where wild or semiwild apes (chimpanzees and gorillas) live, provided valuable preliminary results about the potential role of *Anopheles vinckei* and *An. moucheti* as candidate vectors of *P. praefalciparum* and *P. vivax* between apes (11). This study was nevertheless insufficient to demonstrate that these mosquitoes were effective vectors involved in transmission in absence of evidence of the presence of infecting stage (sporozoite) in salivary glands, and also because of the low number of infected mosquitoes found ($n = 3$).

In the present paper, we present the results of a 15-mo-long longitudinal entomological survey we undertook in two natural parks of Gabon (central Africa) to characterize the sylvatic anopheline species, to identify among them those involved in ape *Plasmodium* transmission and to assess the intensity and dynamics of transmission and their potential as ape-to-human bridge vectors.

Results

Diversity of Trapped Anopheline Mosquitoes. A total of 2,415 female anopheline mosquitoes were caught all along the survey in both study sites (the park of La Lékédi and the national park of La Lopé; Fig. S1). The majority of the specimens have been assigned to 18 known anopheline species. For the remaining specimens ($n = 62$), species could not be determined confidently using morphological and Cytochrome Oxydase Subunit II (COII)-based phylogenetic analyses. In the park of La Lékédi, 1,885 and 231 specimens were respectively collected from the three subsites and around the orphan sanctuaries. These specimens belonged to 15 known species, of which the most frequent were *Anopheles marshallii* (51.18%), *An. moucheti* (22.59%), and *An. vinckei* (14.65%). The number of specimens trapped per anopheline species is given in Table S1. In the park of La Lopé (Mikongo), 299 specimens were trapped all along the survey. They belonged to 10 known anopheles species, among which the dominant species were *An. vinckei* (46.15%), *Anopheles carnevalei* (18.73%), and *Anopheles implexus* (9.03%; Table S1).

Vectors of *Plasmodium*. In total, 2,415 whole bodies and 1,759 salivary glands of *Anopheles* specimens were screened for the presence of *Plasmodium* parasites using a Cytochrome *b* (*Cyt-b*)-based nested PCR method. Eighty were positive (62 whole bodies and 18 salivary glands; Table S1). Overall, nine known *Anopheles* species and one undetermined *Anopheles* species were found infected (Table S1). The average prevalences of *Plasmodium*-infected *Anopheles* whole bodies were 5.02% in La Lopé (Mikongo), 2.06% near the orphan sanctuaries, and 2.17% in the wild sites of La Lékédi (Table S1). The average sporozoite rates in these sites were 1.45%, 0.96%, and 0.94%, respectively.

Alignment of the 80 parasite *Cyt-b* sequences with the references and phylogenetic analyses revealed that they belonged to five major clades (Fig. 1). Four of them match with known parasites infecting several mammal host species (apes, rodents, bats) and one corresponds to parasites recently found in African antelopes (14). Among the sequences belonging to the known parasite clades, some (clades 1 and 2) come from parasites of great apes (57.5%), including parasites from the subgenus *Plasmodium* (25% of *P. vivax*-like, 12.5% of *P. malariae*-like) and from the subgenus *Laverania* [11.25% of *P. adleri*, 3.75% of *P. reichenowi*, 3.75% of *P. praefalciparum*, and 1.25% of *P. gaboni*; from (clade 3) parasites of rodents (5.0% of *P. vinckei* and 5.0% *Plasmodium yoelii*); and from (clade 4) parasites of bats (1.25% of *Polychromophilus* spp.)]. The remaining clade (clade 5) encompassed 31.35% of our sequences, and includes only one known reference of a parasite isolated from an African monkey (15). This clade was recently shown to be mostly composed of parasites of African Bovidae (14).

The details of *Anopheles* species found to be infected by ape *Plasmodium* (as well as by the other parasite clades) are presented in Fig. 1 and Fig. S2. Over all sites, only three species were detected positive to ape parasites: *An. vinckei*, *An. moucheti*, and *An. marshallii*. *Plasmodium* infections were the highest for *An. vinckei*, followed by *An. moucheti* and *An. marshallii* (Fig. S2). For each of these mosquito species, at least one ape parasite was detected in their salivary glands, hence confirming their role as effective vectors of these parasites (Table S2). A power test was performed to determine if other anopheline species could also be involved in transmission of ape *Plasmodium* but remained undetected because of too-small sample sizes. Considering a prevalence of infection similar to the one observed in *An. vinckei* (the highest prevalence recorded in each site), our test revealed that a potential role in ape *Plasmodium* transmission of all other *Anopheles* species could not be excluded ($P > 0.05$).

Factors Affecting the Transmission of Ape *Plasmodium*. A logistic regression was performed to analyze the effects of different factors on the prevalence of ape *Plasmodium* infections in the mosquitoes. Two models were fitted independently. The first model included as fixed factor only the *Anopheles* species. The second model included the height of trap and the month of collection. In this second model, the mosquito species was not included to increase the power to detect a link between ecological factors and the infection by ape *Plasmodium*. Results (Table S3) indicated that all factors had a significant effect (or marginally significant, for height) on transmission. Mosquito infection rates were significantly higher (*i*) for *An. vinckei* than for *An. moucheti* and *An. marshallii*; (*ii*) at midheight than on the ground or at canopy level, and (*iii*) during the rainy season than during the dry season (Fig. S3).

Genetic Structure of Ape *Plasmodium* Vector Populations. To assess the genetic diversity and to search for a potential structure at an intraspecific level among *An. vinckei*, *An. moucheti*, and *An. marshallii* populations, we constructed a phylogeny based on *COII* sequences (Fig. S4). The phylogeny showed low levels of genetic diversity for *An. moucheti* and *An. marshallii* and a higher level for *An. vinckei.* For all vector species, we did not found any evidence for the presence of cryptic species or the existence of genetic structuration associated with the parasite host tropism (gorilla or chimpanzee; Fig. S4).

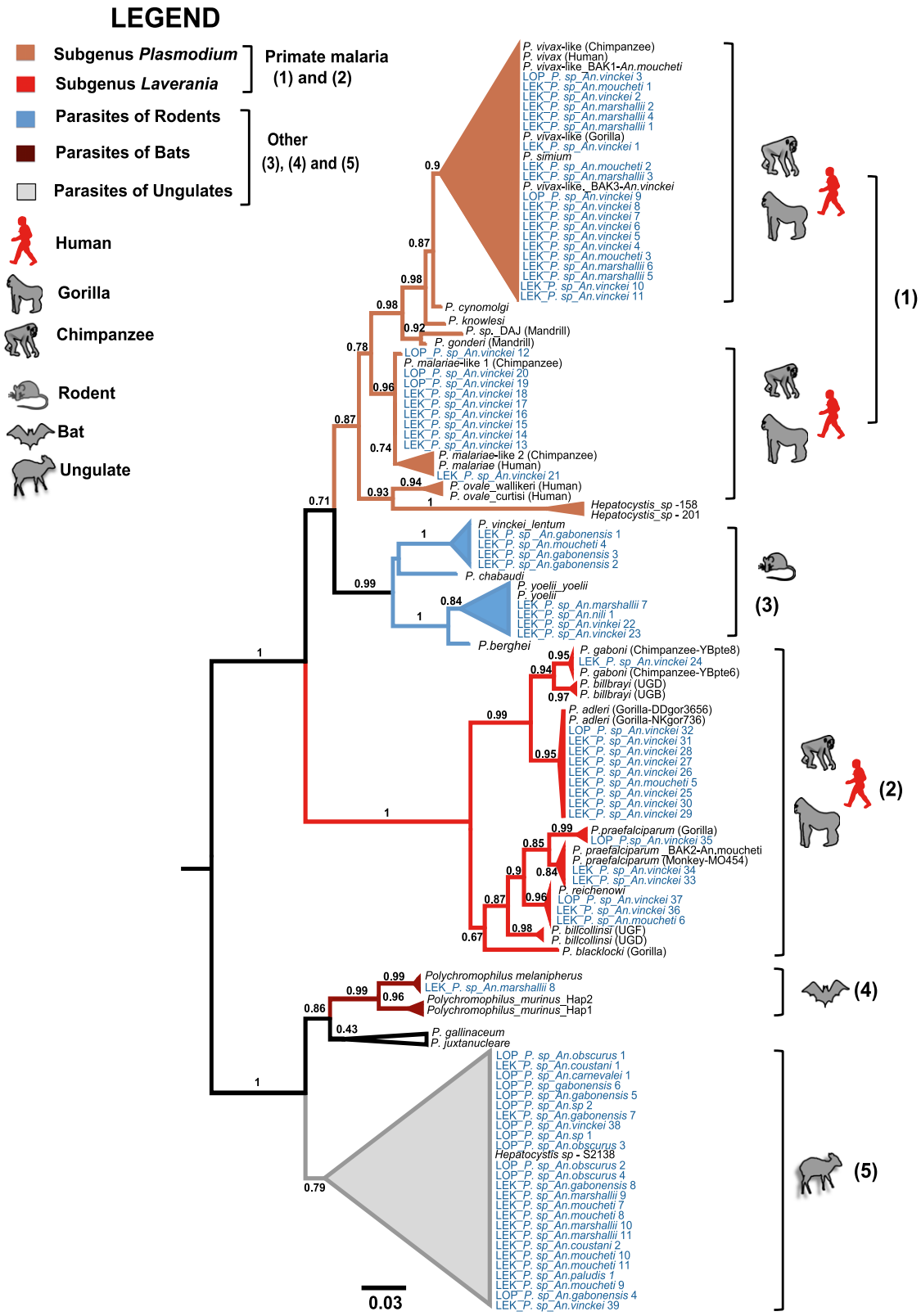

Fig. 1. Phylogenic position of Haemosporidia infecting *Anopheles* mosquitoes in this study. The tree was constructed by using partial *Cyt-b* sequences (773 bp) using maximum-likelihood (ML) methods. Bootstrap values are given at each node. Sequences obtained in this study are in blue.

Tropism of Sylvan Anopheline Mosquitoes Toward Humans. The propensity of sylvan anopheline mosquitoes to feed on humans was tested by using human landing catches. A total of 395 mosquitoes belonging to two genera (*Anopheles* and *Aedes*) were collected. The *Anopheles* genus was well represented, with 99.24% ($n = 392$) of the specimens belonging to six known and one undetermined species

(referred here as *An.* sp.): *An. marshallii* (49%), *Anopheles paludis* (37.5%), *An. moucheti* (11.5%), *An. vinckei* (0.8%), *Anopheles cinctus* (0.3%), *Anopheles gabonensis* (0.3%), and undetermined specimens (0.8%). Over all specimens, two were positive for *Plasmodium* infections and belonged to the species *An. vinckei*. One was infected with a *P. malariae*-like parasite, the other by a *P. vivax*-like parasite.

Discussion

To identify the vectors of ape *Plasmodium* and determine whether they can be involved in the strong host specificity observed in the Laveranias, we performed a longitudinal survey of the anopheline communities in two wildlife reserves of Gabon (central Africa).

Over the entire survey, only three species of anopheline mosquitoes were detected positive to ape *Plasmodium*: *An. vinckei*, *An. moucheti*, and *An. marshallii*. Their role in transmission was confirmed by the detection of the parasites in the salivary glands of several specimens (Table S2). All species were shown to be able to transmit both parasites of chimpanzees and gorillas, and, for one of them (*An. vinckei*), we also have evidence that it can support the transmission of several parasites of different hosts (gorilla and chimpanzee) simultaneously (i.e., same period and same site). Our results therefore suggest that the strong host specificity observed in the Laveranias is not linked to a specific association between the vertebrate host and the vector species but could be caused, as previously suggested, by some more or less permeable genetic barriers involving specific host-parasite ligand/receptor interactions (5, 8).

Beyond *Laveranias*, our study also confirms the high level of transmission among apes of *Plasmodium* species belonging to the subgenus *Plasmodium* and closely related to other human parasites like *P. vivax* or *P. malariae* (2, 7, 16). The same three vector species (*An. vinckei*, *An. moucheti*, and *An. marshallii*) are involved in their transmission. The host specificity of these parasites seems not as strong as that observed for parasites from the *Laverania* subgenus, as *P. vivax*-like and *P. malariae* were found in chimpanzees and gorillas in addition to humans. Mechanisms explaining the stronger switching capacities of non-Laveranias remain poorly understood, but the lack of host specificity of vectors probably favors the phenomenon.

Factors Affecting Infection Rates in Sylvan *Anopheles*. Among the three species that were shown to be involved in ape *Plasmodium* transmission, the highest prevalences of infection were observed for *An. vinckei*, followed by *An. moucheti* and *An. marshallii*. All other collected sylvan species might also be involved in the transmission of ape *Plasmodium* parasites, but our sampling effort was not sufficient to reach a conclusion. Variations among vector species of ape *Plasmodium* infection rates could be explained by several nonexclusive factors, including their trophic behavior, their density, their longevity, their tropism for certain forest microhabitats (e.g., clearing, savannah, or undergrowth), and their susceptibility to infections (17).

Regarding their trophic behavior, very few data are available on these different vectors in strict nonanthropized forest environments. In our study, the three vector species were found infected by a variety of parasite species known to infect different groups of mammals like rodents (*P. yoelii* and *P. vinckei*), ungulates (*Plasmodium* spp.), bats (*Polychromophilus* spp.), and primates, indicating that these vector species bite a broad range of hosts. Moreover, the *COII*-based phylogeny of *An. vinckei*, *An. moucheti*, and *An. marshallii* provided no indication for the existence of population substructure that could be related to differences in host tropism at an intraspecific level. Overall, our results suggest that these vectors are opportunistic rather than specialized for their blood meal. Such a propensity to bite a wide range of hosts is probably an adaptive trait in response to temporal fluctuations of diversity and density of hosts available in the forest. For *An. vinckei*, however, the proportion of ape parasites relative to the other parasites was far higher than for *An. moucheti* or *An. marshallii*, suggesting a possible preference to feed on these hosts when available.

Habitat preference could also explain some of the variations of infection rates between species. Very little is known of the ecology of *An. vinckei* and its habitat preferences. From our data, it seems that this is the dominant species in dense forest environment as in the park of La Lopé (site of Mikongo) or in certain sites of La Lékédi (e.g., site 1). In other places, such as the park of La Lékédi, where forest alternates with savannah (i.e., savannah–forest mosaic), *An. marshallii* predominates, followed by *An. moucheti* and finally *An. vinckei*. Although *An. moucheti* is also known to be a forest vector, *An. marshallii* seems to prefer nonforested areas (18), which may therefore explain its lesser implication as an ape *Plasmodium* vector given that apes generally nest in forest areas.

A higher longevity of *An. vinckei* compared with the other *Anopheles* species could also explain the high infection rate observed in this species despite its lower density in certain environments. Indeed, the longer a mosquito lives, the higher its odds to have several blood meals, which, in turn, increases the probability to become infected (19, 20), but also to allow the complete development of the parasite and its subsequent transmission to a novel host.

Finally, *Anopheles* species may differ in their ability to support the parasite development. This susceptibility, which may vary from complete receptiveness, whereby all individuals support infection, to the opposite, total refractoriness, whereby no individuals support infection, is generally specific of a parasite/vector species combination (21, 22). Thus, a degree of compatibility exists between parasite and mosquito, and the extent of this compatibility determines the success of transmission (23). Past experiments with a chimpanzee infected with *P. reichenowi* showed that some species of *Anopheles* were susceptible to the parasite whereas other species could not be infected, suggesting that not all vectors are able to transmit ape *Plasmodium* (17, 21, 22). Thus, *An. vinckei* could be the most susceptible vector of ape *Plasmodium*.

Beyond the nature of the vector, infection prevalence in sylvan mosquitoes was strongly affected by seasons, with peaks of transmission occurring during the rainy seasons (i.e., from October to December and from February to May), as frequently observed in human foci (24). This seasonality can be explained by the fact that, as rain increases, the number of suitable larval breeding sites for *Anopheles* increases. For *An. vinckei*, breeding sites are creeks with clear water and very shaded places (18). For *An. moucheti*, breeding sites are along the borders of slow-moving streams and large rivers, or in pools and ponds (12, 25). Finally, breeding sites for *An. marshallii* are fresh, clear, shaded water in nonforested environments (18). As the number of breeding sites increases, so does the *Anopheles* population size, which results into an increased malaria transmission (26). Increased temperatures during rainy seasons are also a factor favoring transmission by reducing the time needed for the parasite to complete its development in the mosquito and by accelerating the development of the mosquito larvae (27–29).

Finally, we showed that height of capture under the canopy also explains variations in the prevalence of infection in anopheline mosquitoes. Transmission was the highest at midlevel (10–12 m) but similar at ground and canopy levels. This could partly be because most nests built by chimpanzees and gorillas fall within this range of heights (between 5 and 15 m) (30), thus favoring malaria transmission. In addition, mosquito flying height was shown to be related to host abundance patterns (31, 32). In La Lopé, *An. vinckei* mosquitoes are more abundant at midheight than at other heights, a distribution that could also be driven by the abundance of great ape nests in this site.

Risk of Interspecies Transfers. Several authors have speculated about the possibility that apes could be a source of new *Plasmodium* infections for humans and vice versa, providing a mosquito species could act as bridge between these different host

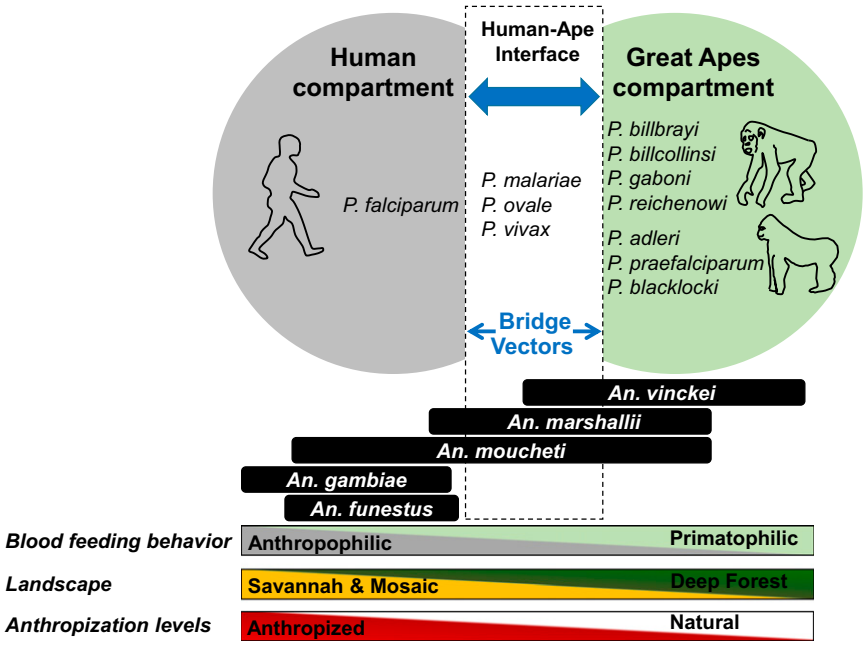

Fig. 2. Schematic view of the role of the *Anopheles* species in the transmission of human and ape malaria parasites and their potential as bridge vectors between the two host compartments according to biological and environmental factors.

species (5, 17). Several recent studies have indeed reported transfers of *Plasmodium* from apes to humans or humans to apes in certain conditions, but the mosquitoes involved in these transfers were never identified (4, 7, 9, 33).

As shown in our study, all three sylvan *Anopheles* species identified as vectors of ape *Plasmodium* (*An. vinckei*, *An. moucheti*, and *An. marshallii*) could act as bridge vectors between apes and humans. Indeed, as shown, all species are opportunistic for their blood meals, and, when available, humans may constitute a possible source of blood, as demonstrated by the human landing catches. In light of our results, it seems that the risk of transfers of parasites to humans could be most important in savannah–forest mosaics where the zoo-anthropophilic vectors *An. marshallii* and *An. moucheti* are present in high densities in addition to *An. vinckei*. Moreover, these risks could be amplified by the presence of human-made structures like ponds or lakes, which could favor the development of *An. moucheti*. Fig. 2 provides a schematic view of the role of the different *Anopheles* species in the transmission of human and ape malaria parasites and their potential as bridges between the two host compartments according to biological and environmental factors.

More generally, our study reveals that sylvan mosquitoes may act as bridges between several groups of mammals, including rodents, primates, ungulates, bats, and humans. Beyond the sanitary risk this may represent, this propensity of sylvan anopheline mosquitoes to feed on different hosts may explain the evolutionary history of *Plasmodium* parasites characterized by numerous host switches that occurred among different mammal groups or species (34–36). Thus, *P. falciparum* in humans likely evolved following a transfer from gorillas (2, 34). Several host switches occurred in the history of the *Laveranias* between gorillas and chimpanzees. Finally, several transfers of parasites likely occurred between bats and rodents as well as between bats and monkeys (37). Therefore, our results highlight the key role played by the feeding behavior of vectors in the switches between hosts and therefore in the diversification of malaria parasites during their evolutionary histories.

Absence of Monkey *Plasmodium*? One surprising result of our study was the absence of *Anopheles* mosquitoes infected by monkey parasites, like *Plasmodium gonderi* or *Plasmodium* sp. DAJ. *P. gonderi* is one of the only two *Plasmodium* species infecting monkeys in west and central Africa (38, 39). Its natural known hosts are the primates of the genera *Cercocebus*, *Mandrillus*, and *Cercopithecus*, well represented in both study sites (La Lopé and La Lékédi). *Plasmodium* sp. DAJ is a *Plasmodium* species that was first discovered in mandrills but that seems to also infect some species of the genus *Cercopithecus* (34). Although nothing is known about the vectors of *P.* sp. DAJ (likely *Anopheles*), previous experimental infections of *Anopheles* specimens have shown their potential implications as vectors for *P. gonderi* (40). The absence of these parasites is surprising, as the density of their hosts is higher than that of great apes in the different parks. One possible reason for this absence could be related to the lack of attractiveness of our traps for their mosquito vectors or to the size of the mosquito sample. Moreover, it is possible that other mosquito species (or arthropods) are involved in the transmission of these parasites. Indeed, the *Plasmodium* of birds are transmitted by mosquitoes belonging to *Culex*, *Aedes*, and *Culiseta* genera, but also by some *Anopheles* and *Mansonia* (41). Other Diptera such as sandflies (Psychodidae), bat flies (Streblidae, Nycteribiidae), black flies (Simuliidae), and Culicoides midges (Ceratopogonidae) are involved in the transmission of several malaria parasites infecting lizards, birds, bats, and monkeys (35, 41, 42).

Conclusion

This study shows that the transmission of *Plasmodium* of great apes would be mainly ensured by *An. vinckei*, *An. moucheti*, and *An. marshallii* in Gabon. Transmission was shown to be highest during the rainy season and at midlevel under the canopy. Because all three species transmit parasites of both chimpanzees and gorillas and do not show phylogenetic structure, our results suggest that the strong host specificity observed in the Laveranias is not linked to a specific association between the vertebrate host and the vector species, but would rather be the consequence of incompatibilities at the parasite/vertebrate interface as previously suggested. In vitro experimental infections of erythrocytes of

different host species by genetically modified parasites could help determine the origin of this host specificity.

Importantly, we also showed that these vector species could assume the role of bridge vectors between apes and humans, as well as with species belonging to other mammal groups. This information is of considerable public health importance as it highlights the possibility of transfers of new zoonotic infections from apes to humans. Although such transfers may be rare as a result of the strong host specificity, they have happened in the past and may be at the origin of new epidemics in humans if some variants are able to cross the species barrier. This is all the more true in a context of reduction of malaria transmission in human populations, which opens new niches for zoonotic parasites. Such propensity of these vectors to feed on different host species should also be of concern for the wildlife conservation community, as potential recurrent release of human infectious diseases to great apes, which are already at high risk of extinction, may accelerate their disappearance.

Materials and Methods

The longitudinal survey of *Anopheles* mosquitoes was performed during 15 mo inside of two wildlife reserves of Gabon. Mosquitoes were identified and screened for the presence of *Plasmodium* parasites by using molecular methods. Generalized linear models were used to explore the effect of ecological factors on *Anopheles* infection rates. *SI Materials and Methods* and Table S4 and S5 provide more details on protocols and methods. Mosquito human landing catches were approved by the national ethic committee of Gabon and informed consent statement was obtained from all participants.

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