Competence of the secondary vectors *An. coustani, An. squamosus* and *An. rufipes* for *Plasmodium falciparum* as measured by direct membrane feeding assays

Domonbabele FdS. Hien[a], Serge R. Yerbanga[a], Dari Y.F. Da[a], Edwige Guissou[a], Bienvenue K. Yameogo[a], Dieudonné D. Soma, Nicolas Moiroux[a,b], Cédric Pennetier[b,c], Karine Mouline[a,b], Kounbobr R. Dabiré[b], Anna Cohuet[a], Thierry Lefèvre[a,b]

[a] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso.
[b] MIVEGEC, IRD, CNRS, Univ. Montpellier, Montpellier, France.
[c] IPR, INSP, Bouaké, Côte d'Ivoire

thierry.lefevre@ird.fr, hiend83@yahoo.fr

Several *Anopheles* species can sustain *Plasmodium falciparum* transmission after the major indoor-resting and human-feeding vectors have been targeted by control interventions such as long-lasting insecticidal nets and indoor residual spraying. The difference in intrinsic susceptibility to *P. falciparum* infection (*i.e.* vector competence) between primary and secondary vector species is usually inferred from natural pattern of infection rates in wild-caught adult females. However besides competence, these patterns depend on various other environmental and genetic factors, including diversity, abundance and accessibility of vertebrate host species, and anthropophilic behaviours. In West Africa, *An. rufipes*, *An. squamosus* and *An. coustani* have been reported to be possible secondary malaria vectors but their intrinsic competence for *P. falciparum* has not been experimentally examined.

F1 progeny of wild *An. rufipes*, *An. squamosus* and *An. coustani* caught in South Western Burkina Faso with calf-baited double net traps were infected with two sympatric field isolates of *Plasmodium falciparum*, using direct membrane feeding assays. Individuals from a laboratory colony of *Anopheles coluzzii* were used as controls. Following the ingestion of the gametocyte-positive bloodmeal, mosquitoes were held in paper cups in standard rearing conditions. Survival was monitored daily until all the mosquitoes had died. From day ten post-infection, the head and thorax of dead mosquitoes were individually stored at -20°C and sporozoite dissemination was assessed using qPCR.

The blood-feeding success on membrane significantly differed among vector species with highest feeding rates observed in *An. coluzzii* (75 ± 6%) followed by *An. squamosus* (24 ± 9%), *An. coustani* (13 ± 5 %) and *An. rufipes* (13 ± 12%). *An. coluzzii* and *An. rufipes* showed similar competence for *P. falciparum* sporozoites (69 ± 14% and 62.5 ± 23.7 %, respectively). However, *An. coustani* and *An. squamosus* were significantly less permissive for the development of *P. falciparum* sporozoites (11 ± 14% and 35 ± 22 %, respectively). There also was a significantly higher quantity of sporozoite DNA in *An. coluzzii* (mean CT= 25 ± 0.95; the lower the CT, the higher is the DNA quantity) than in *An. coustani* (mean CT= 27 ± 1), *An. rufipes* (mean CT= 27 ± 1) and *An. squamosus* (33.3 ± 0.7). Finally, mosquito longevity in laboratory conditions significantly varied among vector species, with best survivorship observed in *An. coluzzii* (mean longevity: 26 ± 1.4 days), followed by *An. rufipes* (20.5 ± 2.15 days), *An. squamosus* (16.2 ± 0.5 days) and *An. coustani* (16 ± 1.1 days).

An. rufipes, *An. squamosus* and *An. coustani* were efficiently infected with *P. falciparum* gametocytes in laboratory conditions. The mechanisms leading to lower competence in *An. squamosus* and *An. coustani* are not yet known but variation in mosquito immune response or blood-meal size could be involved. Because *An. rufipes* displayed relatively long lifespan and high competence for *P. falciparum*, it has the potential to ensure robust transmission, provided that it can feed on humans in natural conditions.