

## Behavior, Chemical Ecology

# Characterization of the swarming behavior of *Anopheles coluzzii* and *Anopheles gambiae* (Diptera: Culicidae) populations in a hybrid zone of Senegal

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*Anopheles gambiae* and *Anopheles coluzzii*, often found in sympatry and synchronous, have undergone a premating reproductive isolation across their distribution range. However, in the Western coast of Africa, unexpected hybridization zones have been observed, and little is known about swarming behavior of these cryptic taxa. Here, we characterized the swarming behavior of *An. coluzzii* and *An. gambiae* to investigate its role in the high hybridization level in Senegal. The study was conducted in the south and central Senegal during the 2018 rainy season. Mating swarms of malaria vectors were surveyed at sunset and collected using an insect net. Meanwhile, indoor resting populations of malaria vectors were collected by pyrethrum spray catches. Upon collection, specimens were identified morphologically, and then members of the *An. gambiae* complex were identified at the species level by polymerase chain reaction (PCR). *An. gambiae* swarmed mainly over bare ground, whereas *An. coluzzii* were found swarming above various objects creating a dark–light contrast with the bare ground. The swarms height varied from 0.5 to 2.5 m. Swarming starting time was correlated with sunset whatever the months for both species, and generally lasted about 10 min. No mixed swarm of *An. gambiae* and *An. coluzzii* was found even in the high hybridization area. These results indicated a premating isolation between *An. coluzzii* and *An. gambiae*. However, the high hybridization rate in the sympatric area suggests that heterogamous mating is occurring, thus stressing the need for further extensive studies.

**Key words:** *Anopheles coluzzii*, *Anopheles gambiae*, hybridization, swarming behavior, Senegal

## Introduction

Despite decades of control efforts, malaria remains a major public health problem worldwide, but affecting mainly the African continent where pregnant women and children under 5 remain the most vulnerable groups (WHO 2021). Furthermore, existing malaria control interventions, including the core vector control tools such as long-lasting insecticidal nets and indoor residual spraying, are facing serious challenges with the widespread insecticide resistance in

natural populations of main malaria vectors (Ranson and Lissenden 2016, Diouf et al. 2020, Gueye et al. 2020). Furthermore, vectors have become more resilient to core indoor interventions among others, behavioral changes, avoiding the contact with insecticide (Pates and Curtis 2005, Moiroux et al. 2012, 2014, Sougoufara et al. 2014), thus leading to the increase of residual malaria transmission (Killeen 2013, Moshi et al. 2018). This situation therefore requires the development of innovative control measures in addition to the

current interventions to reduce malaria transmission and to drive toward malaria elimination goal. So far, alternative control methods such as genetically modified mosquitoes and the release of sterile males are likely the most promising strategies to achieve the elimination goal (Ito et al. 2002, Dame et al. 2009). However, prior to their application, both approaches require a fine understanding of the biology of the targeted species, especially their reproductive behavior and genetic structure (Assogba et al. 2010, Lanzaro and Lee 2013, Diabate and Tripet 2015).

Throughout the sub-Saharan Africa, including Senegal, 3 of the known sibling species of *Anopheles gambiae* s.l. (*An. gambiae*, *An. Coluzzii*, and *An. arabiensis*) play a major role as malaria vectors across their distribution range (Coetzee et al. 2013, Niang et al. 2018, Sy et al. 2018). Indeed, the complex includes 10 morphologically indistinguishable sibling species including *An. arabiensis*, *An. gambiae*, *An. coluzzii*, *An. melas*, *An. merus*, *An. quadrimaculatus*, *An. amharicus*, *An. comorensis*, *An. bwambae*, and *An. fontenillei* (Barrón et al. 2019) which emerged through an ecological speciation process. This ecological divergence likely seems to be still ongoing with the recent description of the incipient species *An. coluzzii* and *An. gambiae* previously known as the M and S molecular forms, respectively (Coetzee et al. 2013). Indeed, recent molecular and genetic studies have shown that, the hybridization rate between the 2 incipient species is extremely rare and lower than 1% when existing in most parts of their distribution area, particularly in the western and central Africa (Torre et al. 2001, Tripet et al. 2001, Sawadogo et al. 2014, Pombi et al. 2017) clearly indicating a reproductive isolation of their natural populations. Then, the 2 species are thought to be separated by a combination of strong mating barriers (Tripet et al. 2001, Diabate et al. 2009, Dabire et al. 2013) and selection against hybrids (Niang et al. 2015). Previous studies on the mating barriers between *An. gambiae* and *An. coluzzii* shows strong assortative mating occurring via spatial swarm segregation (Diabate et al. 2009, Sawadogo et al. 2014) and, within swarms, by short-range recognition mechanisms. Wing beat frequency producing specific flight-tones (Baeshen 2022) and cuticular hydrocarbons acting as contact pheromones (Adams et al. 2021) are thought to play an important role in these mechanisms. However, along the Western coast of Africa, several hybrid zones where gene flow is higher (Oliveira et al. 2008, Niang et al. 2014; Caputo et al. 2016), have been shown suggesting either reduced selection pressure against hybrids or a different mating behavior. At present, higher than expected hybridization rate is well demonstrated, but little is known about swarming behavior of these cryptic taxa in the Western coast of Africa.

The studies of the swarming behavior of *An. coluzzii* and *An. gambiae* have shown strong premating barriers between the 2 species through a disruptive selection schema with segregate swarming sites (Diabate et al. 2003, Poda et al. 2019). While *An. coluzzii* exploits various markers for swarming, *An. gambiae* is mostly found on bare ground (Diabate et al. 2009, 2011). The disruptive selection also act at different swarming heights and times through which heterogamous mating is normally avoided between *An. gambiae* and *An. coluzzii* partners (Charlwood and Jones 1979, Sawadogo et al. 2014). However, the genetic isolation can be sometimes broken leading to heterogamous mating between *An. gambiae* and *An. coluzzii* into few mixed swarms (Diabate et al. 2006, Dabire et al. 2013, Sawadogo et al. 2014). Nevertheless, the rates of mixed mating pairs and interspecific inseminations recorded in natural swarms were very low or inexistent, suggesting an assortative mating between the 2 species (Dabire et al. 2013, Niang et al. 2015). Moreover, an ecological divergences in the larval habitat segregation and the avoidance of predators between the 2 species

have also been well demonstrated (Diabaté et al. 2008, Gimonneau et al. 2012, Niang et al. 2020). Over the year, *An. gambiae* peaks at rainy season and is more associated with temporary rain-dependent larval breeding sites while *An. coluzzii* predominates more in most arid areas colonizing preferentially semi-temporary and temporary breeding sites such as rice fields (della Torre et al. 2005; Lehmann and Diabate 2008, Diabaté et al. 2008).

Whatever the mechanisms involved in the reproductive isolation between *An. coluzzii* and *An. gambiae*, it is likely broken at the westernmost western African region compared to the other regions over their distribution range (Oliveira et al. 2008, Nwakanma et al. 2013, Niang et al. 2014, Caputo et al. 2021). Indeed, in the “Far west Africa”, higher than expected levels of hybrid frequencies have been reported, with 7% in Senegal (Niang et al. 2014), over 7% in Gambia, and 19–24% in Guinea Bissau (Caputo et al. 2008, Oliveira et al. 2008). This suggests the existence of pre and/or postmating factors increasing interspecific mating between *An. coluzzii* and *An. gambiae*. One of the prevailing hypotheses is that different swarming behaviors favoring heterogamous mating in the “Far west Africa” explain the higher than expected gene flow between the 2 species (Nwakanma et al. 2013, Niang et al. 2014).

The current study was undertaken to characterize the swarming behavior of *An. coluzzii* and *An. gambiae* across their sympatric versus allopatric distribution range in the south and central regions of Senegal to unravel potential factors breaking the reproductive isolation between the 2 species.

## Materials and Methods

### Study Area

Three health districts were selected in the center (Fatick) and the south-east (Tambacounda and Kedougou) of Senegal. In each of the selected district, 2 villages were chosen based on their distinctive ecological and entomological features (Fig. 1).

The villages of Kouar (13°18'59.6"N, 13°33'50.6"W) and Sare Sidy (13°25'18.9"N, 13°40'48.1"W), located in the health district of Tambacounda, were selected as a sympatric area of *An. coluzzii* and *An. gambiae* with higher than expected level of hybridization (0.7–6.7%) compared to elsewhere in the country (Niang et al. 2014, 2016). The study area is located next to the Gouloumbou River, in a wetland characterized by tree-shrubby savannah and irrigated cultivated landscapes, with the presence of numerous large and permanent water bodies as previously described (Niang et al. 2014, 2016). The climate is Sudanian, with a rainy season lasting from June to October, during which temperatures are irregular, averaging around 28 °C (ANSD 2018).

The localities of Silly (12°32'33.1"N, 12°16'14.1"W) and Bandafassi (12°32'18.6"N, 12°18'35.4"W) located in the south-eastern health district of Kedougou, were chosen as an allopatric area (or area of predominance) for *An. gambiae* (Fall 2016). Both health districts belong to the Sudan-Guinean climatic domain, where the rainy season lasts from June to October with a peak in August–September.

In the third health district of Fatick, the localities of Toubanding (13°43'29.7"N, 16°25'24.9"W), and Passy (13°41'56.2"N, 16°23'44"W) were selected as an allopatric area (or area of predominance) for *An. coluzzii* (Niang et al. 2014, Gueye et al. 2020). The area is in the coastal zone of the country belonging to Sudan-Sahelian climatic domain where the rainy season lasts from June to October with low and irregular rainfall. The study area has the particularity of being crossed by the river of Nema, creating a specific microenvironment characterized by the presence of permanent mosquito larval sites.

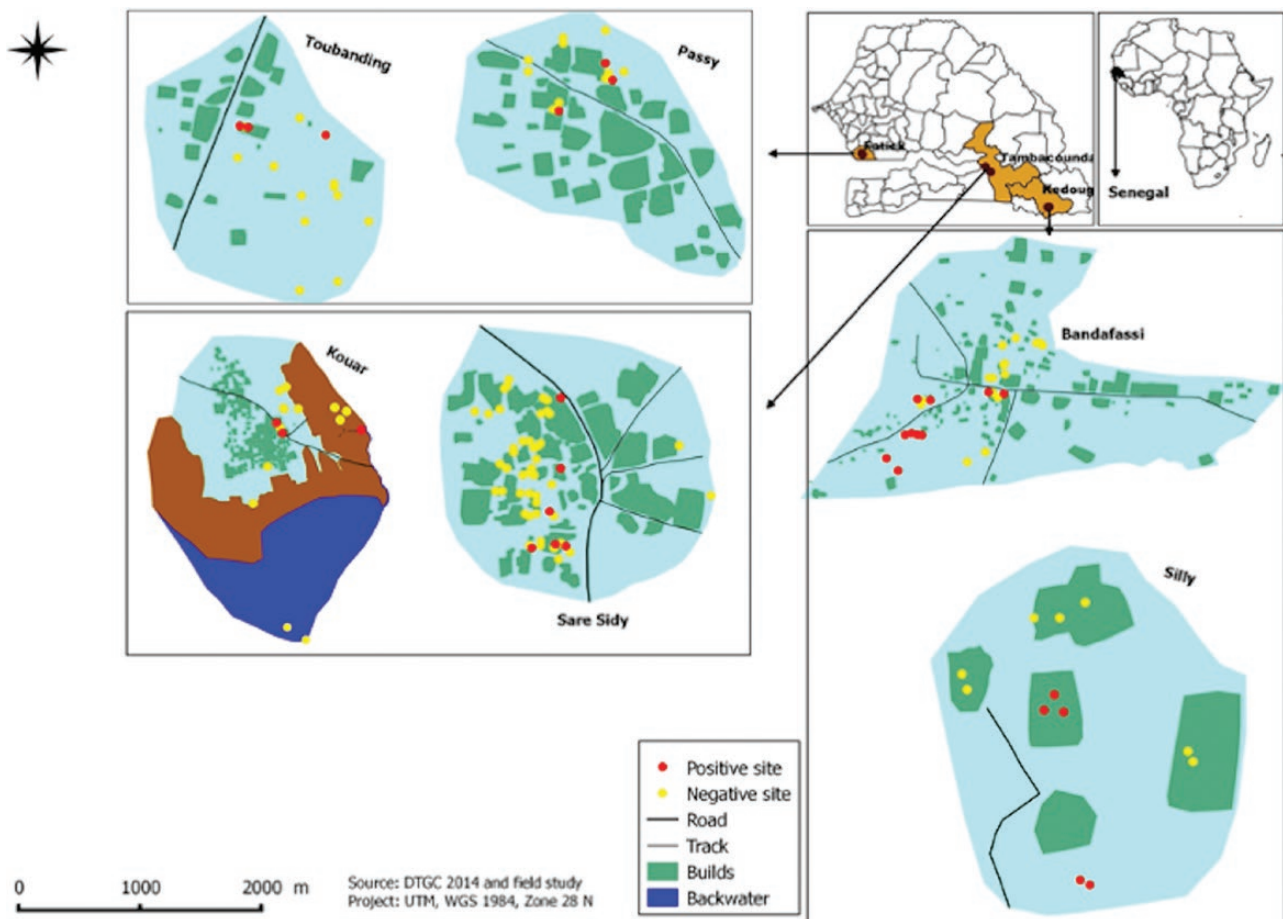


Fig. 1. Putative swarm locations of *An. gambiae s.l.* species surveyed over the time in each of the study areas.

### Swarms Survey and Collection

The study was conducted during the rainy season from August to October 2018. Swarm's observations were conducted during 3 successive days per month in each of the study villages except for Kourar and Sare Sidy in the Tambacounda health district, where observations were missed in the month of September due to logistical issue. Swarm observers and collectors were hired and trained to carry out the survey and mosquito collection from the identified swarm. Once a specific or potential swarm marker is located throughout the villages, it was followed up every evening over 3 successive days. Once a swarm is detected, its marker locations is georeferenced using a global position system with 2 m positional accuracy and mapped using the QGIS 3.4 software version. Swarm observations started at sunset and was performed by observers posted towards and checking the lightest part of the sky from 0.5 to 4 m above the ground level. Once located, with the apparition of the first male known as the precursor, the size of the surveyed swarms was evaluated ( $10 \pm 2$  min after), and the approximate number of swarming males was estimated. The height of each swarm from the ground was estimated using a rod graduated from 1 to 3 m. The number of mating pairs were counted as soon as spotted as they leave the swarm forming a bigger dot easily visible with naked eyes. Swarming males and mating pairs were sampled from swarms using a net as described previously (Diabate et al. 2009), aspirated from the net and transferred into a cup containing cotton soaked with chloroform to kill them. Then, species were identified morphologically and kept individually in labeled 1.5 ml Eppendorf tubes with silica gel desiccant.

During the survey, parameters related to the swarming behavior were recorded, including the swarm marker, the start and end times of swarming activity, swarm size, and height above the ground. The sunset times were recorded in the field and confirmed on <https://www.sunrise-and-sunset.com> website every day for each study site. The swarming beginning time of each swarm was also recorded in each of the study sites and a simple correlation was done between these 2 parameters. The collected specimens morphologically identified as *An. gambiae s.l.*, were further identified in the laboratory by polymerase chain reaction (PCR) to distinguish the complex sibling species (Wilkins et al. 2006).

### Indoor Resting Collection

Pyrethrum spray collection was performed in the morning of the second day of the survey in 10 randomly selected human dwellings nearby the identified swarming sites in each of the study villages to estimate the relative frequency of *An. coluzzii*, *An. Gambiae*, and their hybrids. The collection was done from August to October, except in Tambacounda where sampling was missed in September as explained above. Upon collection all specimens were morphologically identified using dichotomous identification keys of Gillies et De Meillon (Gillies and De Meillon 1968), stored individually in 1.5 ml Eppendorf tubes with silica gel then subsequently identified to species level by PCR (Wilkins et al. 2006).

### Characterization of Swarming Markers

Since previous studies have shown that each *An. gambiae* species mate in flight over a specific physical objects that contrast with

the ground or over the ground (Diabate et al. 2009), all previously described potential swarming landmarks have been identified and recorded on the first day of this current survey in each of the study villages. Then, these recorded putative swarming markers were subsequently monitored during the sunset over 3 consecutive days per month. Once positive, the markers were recorded, geolocated, and further characterized regarding the type of landmark, the times of swarm initiation and dispersal as well as its duration, the swarm size and height above the landmark to describe the swarming behaviors of each of the studied incipient species in across their sympatric versus allopatric zone in Senegal. To better understand the role of physical markers for swarm site selection by the different species, positive swarm markers were photographed and characterized as described in previous works (Diabate et al. 2009, Assogba et al. 2014, Sawadogo et al. 2014). The visual markers used by mosquito males for swarming in different sites were split into 5 types including, bare ground, grass, wood, waste and well (Fig. 3A).

### Data Analysis

Swarm height, duration, and markers were compared between *An. coluzzii* and *An. gambiae* using the Wilcoxon test and Student's *t*-test. Their sizes were estimated at the swarming peak and compared between the 2 species and study areas. Swarm duration was calculated as the lag time in minutes between the starting time and the ending time for each observed swarm.

Statistical analysis and correlation tests using the Kendall correlation test were performed with a significance level of 0.05. All the statistical analysis were done using the R 3.5.2 software version.

## Results

### Location of Swarming Sites in the Study Areas and Swarm Collection

Geographic coordinates used to map the swarming sites in the 3 study districts of Kedougou, Tambacounda, and Fatick showed that

the distribution of swarming sites was not homogeneous throughout the study villages but rather aggregated at specific locations (Fig. 1). Remarkably, swarming likely occurs either inside (11%) or outside (89%) the courtyards, and only within the boundaries of the villages where they were observed (Fig. 1).

Overall, 197 males were collected from 32 swarms across different areas. Of these, 22 were specific to *An. gambiae*, 6 to *An. coluzzii*, and 4 to *An. arabiensis*. Noteworthy, no mixed swarm was found over the area during the study period (Table 1).

In the district of Kedougou, chosen as the allopatric area of *An. gambiae*, only swarms of *An. gambiae* were found during the study period. These consisted of 15 swarms in Kedougou, 10 in Bandafassi, and 5 in Silly. Conversely, 4 swarms with only *An. coluzzii* were recorded in Passy (Fatick), selected as an allopatric area for this species. While in the sympatric area of Tambacounda, swarms of both species were found in Kouar with 1 of *An. gambiae* and 2 of *An. coluzzii*. However, in the study village of Sare Sidy, only *An. gambiae* swarms ( $n = 5$ ) were found.

Over the time, a temporal variation in the number of swarms was noted, the highest numbers were observed in August and September while the lowest number was recorded in October except in Kouar (Tambacounda) where swarms were only found in October (Table 1).

### Species Composition of the Indoor Resting Populations

Overall, 819 specimens of *An. gambiae* s.l. were collected resting inside the randomly selected human dwellings in each of the study site of the 3 areas. Subsequent molecular analysis showed that *An. arabiensis* was found in all the different study areas and was mostly predominant in Fatick, while *An. melas* was only found in Fatick (Table 2).

Contrary to the swarming populations, the resting populations in Kedougou and Fatick revealed the concomitant presence of resting *An. gambiae* and *An. coluzzii* specimens. The hybrids of the 2 species were found in Passy (Fatick) and Tambacounda. In the

**Table 1.** Monthly number of collected swarms and their species composition in different villages of Tambacounda, Kedougou, and Fatick from August to October 2018 except September in Tambacounda

Areas	Villages	Mois	Number of swarms			
			<sup>a</sup> <i>An. arabiensis</i>	<sup>a</sup> <i>An. coluzzii</i>	<sup>a</sup> <i>An. gambiae</i>	<sup>b</sup> Mixed
Tambacounda	Kouar	August	0	0	0	0
		September	–	–	–	0
		October	0	2	1	0
	Sare Sidy	August	0	0	5	0
		September	–	–	–	0
		October	1	0	1	0
Kedougou	Bandafassi	August	0	0	2	0
		September	0	0	6	0
		October	0	0	2	0
	Silly	August	0	0	4	0
		September	0	0	1	0
		October	0	0	0	0
Fatick	Toubanding	August	3	0	0	0
		September	0	0	0	0
		October	0	0	0	0
	Passy	August	0	0	0	0
		September	0	3	0	0
		October	0	1	0	0

<sup>a</sup>Monospecific swarms comprised of *An. arabiensis*, *An. gambiae* or *An. coluzzii* individuals.

<sup>b</sup>Mixed swarms comprised of at least 2 species individuals.

region of Kedougou, *An. gambiae* was the most predominant species representing 87.5% in Bandafassi and 92.7% in Silly compared to its sibling species, *An. coluzzii* which represents only 12.5% in Bandafassi and 7.3% in Silly (Table 2). Conversely, in Fatick, *An. coluzzii* represented 90% of the resting populations in Toubanding and 88.3% in Passy, compared to *An. gambiae* which consisted 10% of specimen collected inside human dwelling in Toubanding and 9.8% in Passy. Interestingly, 1.9% of hybrids were found in Passy (Table 2). In the sympatric area of Tambacounda, a higher hybridization rate was confirmed being the highest in Kouar (4.4%), followed by Sare Sidy (2.4%), where the proportions of the 2 incipient species were for *An. gambiae* 60.2% in Kouar and 83.4% in Sare Sidy, and for *An. coluzzii* 35.4% in Kouar and 14.2% in Sare Sidy (Table 2). Over the time, no *An. coluzzii* was collected in Kedougou during the months of August and October (Fig. 2A) as well as for *An. gambiae* in Toubanding (Fatick) (Fig. 2C). However, in both villages in Tambacounda and Passy both *An. gambiae* and *An. coluzzii* were found over the whole study period, with a maximum of 2 *An. gambiae* in Passy (Fig. 2B and C). The highest vectors densities were recorded in the month of September both in the localities of Kedougou and Fatick, excepted for *An. gambiae* in Silly, where the number of the collected individuals decreased during that month.

## Type of Swarm Markers

In this study, the main types of visual markers over which swarms were regularly formed were 5 (Fig. 3A). In Kedougou, the main swarm marker used by males of *An. gambiae* during the study period was the bare ground (74%) followed by other physical markers including grasses (13%) and wood piles (13%) (Fig. 3B). On the other hand, the swarm markers used by *An. coluzzii* males, exclusively found in Fatick, were constituted by physical markers such as wastes (50%), wood piles (25%), and grasses (25%) (Fig. 3B). Finally, in Tambacounda, where both species were found in sympatry, *An. gambiae* males swarmed over bare ground while those of *An. coluzzii* used both bare ground (50%) and wells (50%) (Fig. 3B).

## Swarm Characteristics (Size, Height, and Duration)

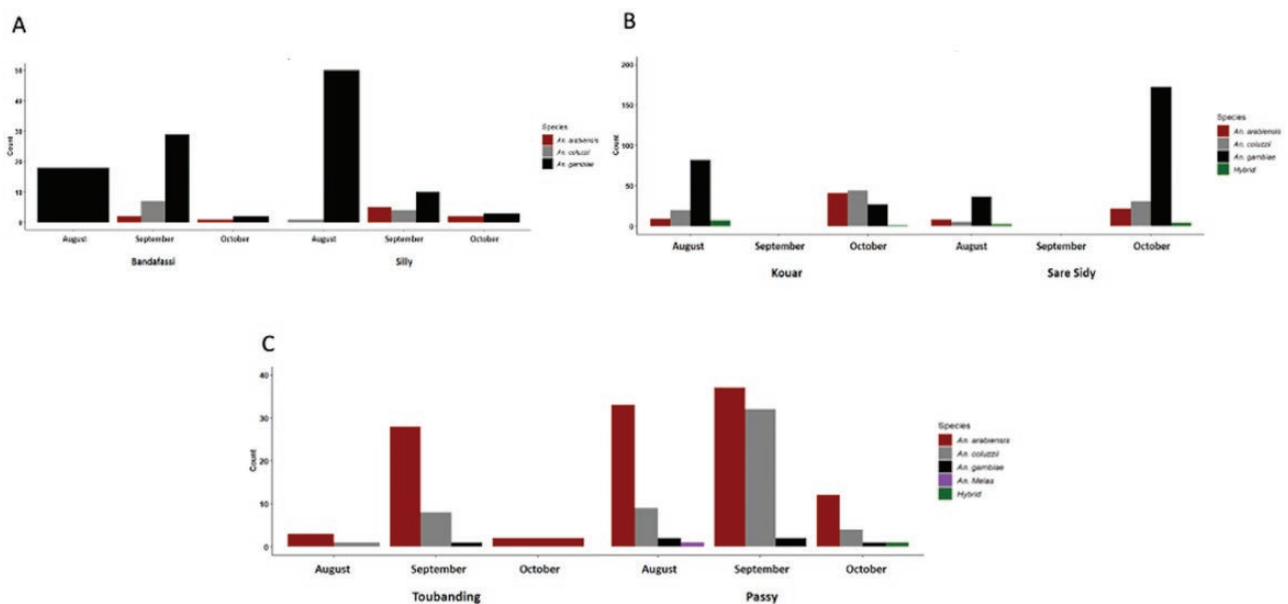
In Kedougou, except 1 swarm made of 100 males, the swarm size of *An. gambiae* varied approximately between 5 and 60 males with a mean of 36 (CI: 22.58–49.41) males per swarm (Fig. 4A). In Fatick, the size of *An. coluzzii*'s swarm varied between 5 and 30 males with a mean of 16.25 (CI: –1.39–33.89) males per swarm (Fig. 4A). While in the sympatric area of Tambacounda, the mean swarm size was 35.71 males (CI: 15.83–55.59) per swarm for *An. gambiae* and 25 males (CI: –165.59–215.59) per swarm for *An. coluzzii* (Fig. 4A).

**Table 2.** Species composition of indoor samples collected in the different villages of Tambacounda, Kadougou, and Fatick during the rainy season in 2018

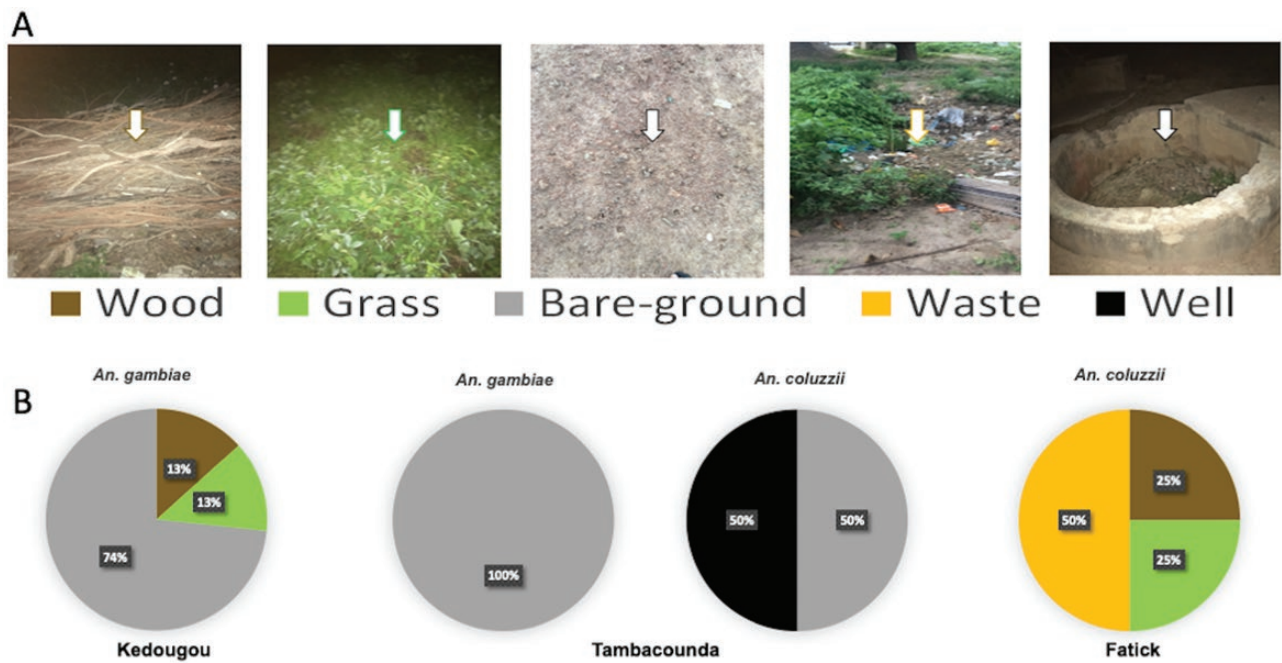
Study areas	Village	<i>An. arabiensis</i>	<i>An. gambiae</i> s.s	<i>An. melas</i>	<sup>a</sup> N	<i>An. coluzzii</i>	<i>An. gambiae</i>	<sup>b</sup> Hybrid
Tambacounda	Kouar	50 (21.65%)	181 (78.35%)	–	231	64 (35.36%)	109 (60.22%)	8 (4.42%)
	Sare Sidy	29 (10.51%)	247 (89.49%)	–	276	35 (14.17%)	206 (83.40%)	6 (2.43%)
Kedougou	Bandafassi	3 (5.08%)	56 (94.92%)	–	59	7 (12.5%)	49 (87.5%)	0
	Silly	7 (9.33%)	68 (90.67%)	–	75	5 (7.35)	63 (92.65%)	0
Fatick	Toubanding	33 (76.74%)	10 (23.26%)	–	43	9 (90%)	1 (10%)	0
	Passy	83 (61.48%)	51 (37.78%)	1 (0.74%)	135	45 (88.3%)	5 (9.80%)	1 (1.9%)

<sup>a</sup>Number of specimens of the *An. gambiae* complex identified at the species level in each village.

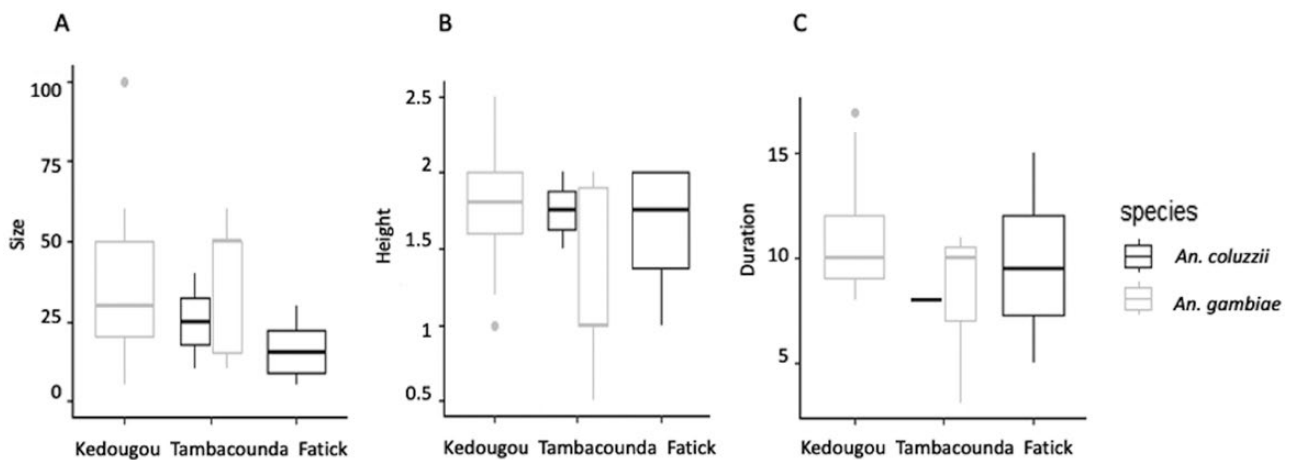
<sup>b</sup>Hybrid individuals between *An. gambiae* and *An. coluzzii*.



**Fig. 2.** Monthly species composition of indoor resting population at study areas from August to October. The bar indicates the monthly total number of individual mosquitoes collected for each species in A) Kedougou, B) Tambacounda, and C) Fatick.



**Fig. 3.** Swarming markers used by *An. coluzzii* and *An. gambiae* across the study area. A) Pictures of the different swarming markers found in the study areas. B) Proportion of each swarming maker by species and study area.



**Fig. 4.** Swarming characteristics of *An. coluzzii* and *An. gambiae* across the study areas. A) Represent the swarm size, B) swarm height, and C) swarm duration.

In Kedougou, the swarm height varied between 1 m and 2.5 m for *An. gambiae* with a mean swarming height of 1.80 m (CI: 1.57–2.04) (Fig. 4B), and in Fatick, it was ranged from 1 m to 2 m for *An. coluzzii* with most of the swarm recorded around 2 m above the ground (Fig. 4B). Finally, in Tambacounda, representing the sympatric area, the swarm height varied from 1.5 m to 2 m for *An. coluzzii* against 0.5 m to 2 m for *An. gambiae*. However, the swarm height of the 2 species were similar ( $w = 10$ ;  $P = 0.45$ ) in this area.

The swarming duration lasted between 7 and 17 min for *An. gambiae* in Kedougou with a median swarming duration of 11 min (CI: 9.22–12.77) (Fig. 4C), however in Fatick, it lasted from 5 to 15 min for *An. coluzzii* with a median swarm duration of 9.75 min (CI: 2.95–16.54). And finally, in their sympatric area of Tambacounda, the median values of the swarm duration of *An. coluzzii* and *An. gambiae*, were respectively 8 and 8.42 min (CI: 5.66–11.19). However, no significant difference ( $t = -0.04$ ;  $dl = 6$ ;  $P = 0.71$ ) was

found between the swarm duration of the 2 species in this sympatric area (Fig. 4C).

#### Correlation of the Sunset and Swarming Beginning Times

Overall, across the study area, swarming started between 2 and 9 min after the sunset, with a significant correlation of the 2 variables in the different areas study: Kedougou ( $\tau = 0.74$ ;  $P < 0.001$ ), Tambacounda ( $\tau = 0.75$ ;  $P = 0.002$ ), and Fatick ( $\tau = 0.70$ ;  $P = 0.01$ ) (Fig. 5).

#### Swarm Composition and Hybridization Level

The hybridization level recorded in Tambacounda, a sympatric area for the 2 incipient species, was 4.4% and 2.4% of *An. gambiae*–*An. coluzzii* hybrids, respectively. Hybridization was also recorded in Fatick (1.9%).

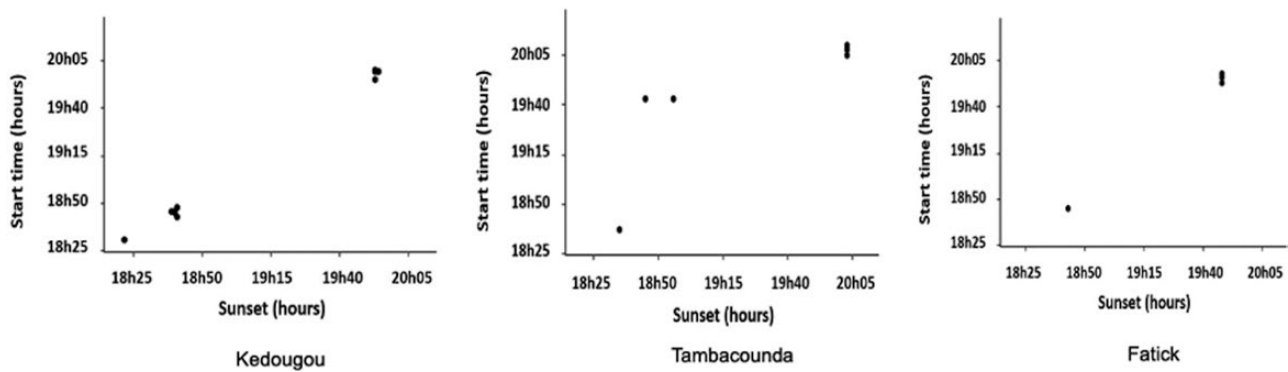


Fig. 5. Relationship between sunset time and the swarming beginning time in Kedougou, Tambacounda, and Fatick.

The finding of higher-than-expected hybridization level in some areas (Tambacounda, 4.4% *An. gambiae*–*An. coluzzii* hybrids) suggests the putative presence of mixed swarms. Noteworthy, no mixed swarm has been found in any of the study site and during the study period, being all 100% monospecific, even in the area of the highest gene flow (Table 1).

## Discussion

In this study, the swarming behavior of *An. coluzzii* and *An. gambiae* was investigated for the first time in Senegal in areas with distinctive ecological features, selected based on previous studies of *An. gambiae* s.l. species distribution (Niang et al. 2014, 2016, Fall 2016).

The study results showed that, except few swarms observed within courtyard in Kedougou, almost all the swarms were found outside but at the close vicinity of the human dwelling. The observed behavior of swarming close by human habitations likely ensure to these species to remain in the immediate environment of the human host allowing females to easily access blood sources, preferably from the human host right after mating for the development of their eggs. This suggest that males likely form swarms mainly where females have immediate access to their hosts after mating and where males find many sources of nectar to replenish their energy reserves after swarming. These results are consistent with previous findings in Tanzania (Marchand 1984), Mali (Diabate et al. 2009), and Burkina Faso (Sawadogo et al. 2014).

The study results revealed the predominance of *An. gambiae* in Kedougou and *An. coluzzii* in Fatick in the indoor collected samples during the study period. Moreover, only a monospecific swarm of *An. gambiae* was found in Kedougou, while the identified swarms found in Fatick were exclusively made of *An. coluzzii*. However, in the sympatric area of Tambacounda the swarms of both species were found. Previous studies have shown that the 2 incipient species display different ecological preferences both at the larval and adult stages (Gimonneau et al. 2012), explaining their spatial and temporal distribution (Touré et al. 1998, Niang et al. 2015). Indeed, Kedougou is characterized by a long raining season with temporary or semi-temporary breeding sites whereas Fatick is an arid area with a short raining season characterized by low and irregular rainfall. In Fatick, the presence of the Nema River which creates micro-ecological condition offering permanent breeding sites for the development of *An. gambiae* or *An. coluzzii* larvae. Notably, *An. gambiae* larvae are mainly found in rain-dependent surface water bodies while those of *An. coluzzii* are more adapted to more permanent anthropogenic breeding sites such as irrigated rice fields (della Torre et al. 2005, Diabate et al. 2005, Gimonneau et al. 2012, Kamdem et al.

2012). Furthermore, even if both species are characterized by a low tolerance of water salinity (White et al. 2013), *An. coluzzii* larvae displayed a greater tolerance to aridity (Kamdem et al. 2012).

*An. gambiae* the major malaria vector in Africa swarms over specific landmarks known as swarm markers (Charlwood et al. 2002, Diabate et al. 2003, 2009, Yuval 2006). The results obtained here support the determinant role of swarm markers in swarm segregation between *An. coluzzii* and *An. gambiae*. Swarm markers exploited by *An. coluzzii* and *An. gambiae* in their respective areas were different. In Kedougou, *An. gambiae* was found swarming mainly over the bare ground and accidentally over dry grasses or wood piles. Conversely, in Fatick males of *An. coluzzii* were found swarming either over grasses, waste, and wood piles, but none over the bare ground. Nevertheless, in the sympatric area, even though *An. gambiae* was found exclusively swarming over the bare ground, *An. coluzzii* did not display the same restrictive preference in terms of swarm marker since it was found both above bare ground and well. Recent studies in Burkina Faso have demonstrated both in laboratory and semi-field conditions that males of the 2 incipient species use different visual markers. With *An. coluzzii* swarming right above physical markers, while *An. gambiae* males use a distant marker but swarm above bare ground (Poda et al. 2019). Unexpectedly, in Kedougou the bare ground swarming site found in August, covered by grasses in September remained the same swarming site used by *An. gambiae*. This suggests that males preferentially occupy these sites, which they recognize either by the presence of objects at a certain distance or by other factors which may be related to the mosquito genetics, or ecology.

Our results showed that swarms were always initiated by 1 or 2 males displaying a tortuous flight pattern as found in other studies (Charlwood et al. 2002, Diabate et al. 2003, 2009, Assogba et al. 2014, 2022, Sawadogo et al. 2014). During swarming, females approach swarms to select a mating partner then leave the swarm with the selected male in copula (Diabate et al. 2003, 2009). In this study, only a few mating pairs was observed, this may be probably due to the low swarm size obtained compared to what was observed in previous studies (Diabate et al. 2003, 2006, Dabire et al. 2013, Sawadogo et al. 2013, 2014, Niang et al. 2015). The small swarm size found may be related to low population densities as reflected by the indoor resting density observed in the study sites. Indeed, studies in Benin have shown the association between indoor resting density and swarm size (Assogba et al. 2014). However, the same pattern of small swarming size with low indoor resting density was found in The Gambia (Assogba et al. 2022), a bordering country of Senegal and close by this study areas. Therefore, more research is needed to further characterize factors likely influencing the swarm size and

the potential contribution of indoor resting density. The association between swarming size and mating events has also been reported in Burkina Faso and Benin, the number of mating pairs is shown to increase with the swarm size (Assogba et al. 2014, Sawadogo et al. 2014, Bimbilé Somda et al. 2018).

The analysis of the swarming and the mating behaviors of *An. gambiae* in the field suggests that mating partners avoid interspecific contact through mainly disruptive swarming height above the ground (Charlwood and Jones 1980). The study results showed that the swarm height varies in the same village likely due to the absence or presence of an obstacle that may impede the opening to the western horizon. Swarm height is shown to be influenced by the opening site to western the horizon. Indeed, next to an obstacle mosquitoes would seek a suitable height from which they could have some view of the western horizon (Sawadogo et al. 2014, Baeshen 2022).

The occurrence of crepuscular swarms of *An. coluzzii* and *An. gambiae* appears to be controlled predominantly by the sunset time over the season, with swarms formed with the reduction of the sunlight (Sawadogo et al. 2013, Wang et al. 2021). The results presented in this study shows that the sunset time is an environmental factor that is strongly correlated with the swarm beginning activity. In fact, regardless of the study area or monthly period of collection, swarms appear always 2–8 min after sunset time. This result match with the previous studies suggesting that a biological clock regulates the timing of swarming activity of the species (Wang et al. 2021) which allows them to adjust themselves to cyclic changes in day length and the timing of sunset through the year.

The comparative analysis of the swarming characteristics, including the swarming height and duration showed no significant difference between *An. gambiae* and *An. coluzzii* when comparing them in their sympatric areas. The same pattern was observed with the swarm markers where bare ground was used by the 2 species. These results suggest that during swarming period, *An. gambiae* and *An. coluzzii* may overlap at time in the sympatric area leading to the possibility of heterogamous mating allowing the occurrence of a high hybridization level even though no mixed swarm was found yet.

The observed high hybridization level without no recorded mixed swarm of *An. gambiae* and *An. coluzzii* could be explained, on one hand by the limited temporal sampling including the missed data in September in the sympatric area of Tambacounda or occurring where and when they were not expected. And, on the other hand, a possibility through indoors mating in the absence of any form of conspecific recognition as demonstrate previously (Dao et al. 2008). Therefore, to link the swarming behavior and hybridization rate, Sawadogo and collaborators which found no hybrids from indoor resting samples or mating pairs despite the high frequencies of mixed swarms suggest that the occurrence or absence of mixed swarms is not necessarily the main pathway for the 2 incipient species hybridization (Sawadogo et al. 2014). Therefore, further investigations need to be undertaken considering all the above hypothesis to better understand the underlying factors of the “Breakdown in the Process of Incipient Speciation in *Anopheles gambiae*” at its furthest “Far-west” distribution range (Nwakanma et al. 2013, Niang et al. 2014).

## Conclusion

This study provides the first evidence on the swarming behavior of the natural populations of *An. coluzzii* and *An. gambiae* in Senegal. The results reported here suggest a possible premating isolation between the 2 incipient species. However, the analysis of the species composition of the resting populations across the study area suggests

that heterogamous mating still occurring during time at place yet to be known. Despite being preliminary, these results are crucial for the design of future studies to better characterize the mating behavior of the 2 incipient species to better support current and future vector control methods, including the release of sterile modified males.

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## Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon request.

## Ethics Approval and Consent to Participate

This study was approved by the Ethics committee of University Cheikh Anta DIOP of Dakar, Senegal.

## Author Contributions

Oumou Gueye (Data curation [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Lead], Writing – original draft [Lead]), Abdoulaye NIANG (Validation [Equal], Writing – review & editing [Equal]), Mouhamed Faye (Data curation [Equal], Investigation [Equal]), Abdoulaye Dia (Formal analysis [Supporting]), Amblat Ahmed (Data curation [Equal], Investigation [Equal]), Ousmane SY (Supervision [Supporting], Validation [Supporting]), Frederic Tripet (Conceptualization [Lead], Supervision [Lead], Validation [Lead]), Lassana Konaté (Validation [Supporting]), Ibrahima Dia (Supervision [Lead], Validation [Lead]), Charles Wondji (Conceptualization [Lead], Supervision [Lead], Validation [Lead]), Oumar Gaye (Funding acquisition [Lead], Validation [Lead]), Ousmane Faye (Supervision [Lead], Validation [Lead]), Abdoulaye DIABATÉ (Conceptualization [Lead], Supervision [Lead], Validation [Lead]), and El H Niang (Conceptualization [Lead], Methodology [Equal], Supervision [Lead], Validation [Lead], Writing – review & editing [Lead])

## References

- Adams KL, Sawadogo SP, Nignan C, Niang A, Paton DG, Robert Shaw W, South A, Wang J, Itoe MA, Werling K, et al. Cuticular hydrocarbons are associated with mating success and insecticide resistance in malaria vectors. *Commun Biol*. 2021;4(1):911. <https://doi.org/10.1038/s42003-021-02434-1>.
- ANSD. Service Régional de la Statistique et de la Démographie de Tambacounda: Situation économique et sociale regionale 2017-2018; 2018.



- Assogba BS, Djogbenou LS, Dabiré RK, Diabaté A, Baldet T. Studies on the breeding swarms of *Anopheles gambiae* complex in malaria control perspective. *Malar J*. 2010;9(S2):O1. <https://doi.org/10.1186/1475-2875-9-S2-O1>
- Assogba BS, Djogbénou L, Saizonou J, Diabaté A, Dabiré RK, Moiroux N, Gilles JR, Makoutodé M, Baldet T. Characterization of swarming and mating behaviour between *Anopheles coluzzii* and *Anopheles melas* in a sympatric area of Benin. *Acta Trop*. 2014;132:S53–S63. <https://doi.org/10.1016/j.actatropica.2013.09.006>
- Assogba BS, Sillah S, Opondo KO, Cham ST, Camara MM, Jadama L, Camara L, Ndiaye A, Wathuo M, Jawara M, et al. *Anopheles gambiae* s.l. swarms trapping as a complementary tool against residual malaria transmission in eastern Gambia. *Sci Rep*. 2022;12(1):17057. <https://doi.org/10.1038/s41598-022-21577-7>
- Baeshen R. Swarming behavior in *Anopheles gambiae* (sensu lato): current knowledge and future outlook. *J Med Entomol*. 2022;59(1):56–66. <https://doi.org/10.1093/jme/tjab157>
- Barrón MG, Paupy C, Rahola N, Akone-Ella O, Ngangué MF, Wilson-Bahun TA, Pombi M, Kengne P, Costantini C, Simard F, et al. A new species in the major malaria vector complex sheds light on reticulated species evolution. *Sci Rep*. 2019;9(1):14753. <https://doi.org/10.1038/s41598-019-49065-5>
- Bimbilé Somda NS, Poda BS, Sawadogo PS, Gnankiné O, Maiga H, Fournet F, Lees RS, Bouyer J, Gilles J, Sanon A, et al. Ecology of reproduction of *Anopheles arabiensis* in an urban area of Bobo-Dioulasso, Burkina Faso (West Africa): monthly swarming and mating frequency and their relation to environmental factors. *PLoS One*. 2018;13(11):e0205966. <https://doi.org/10.1371/journal.pone.0205966>
- Caputo B, Nwakanma D, Jawara M, Adiamoh M, Dia I, Konate L, Petrarca V, Conway DJ, della Torre A. *Anopheles gambiae* complex along The Gambia river, with particular reference to the molecular forms of *An. gambiae* s.s. *Malar J*. 2008;7:182. <https://doi.org/10.1186/1475-2875-7-182>
- Caputo B, Pichler V, Bottà G, De Marco C, Hubbart C, Perugini E, Pinto J, Rockett KA, Miles A, Della Torre A. Novel genotyping approaches to easily detect genomic admixture between the major Afrotropical malaria vector species, *Anopheles coluzzii* and *An. gambiae*. *Mol Ecol Resour*. 2021;21(5):1504–1516. <https://doi.org/10.1111/1755-0998.13359>
- Caputo B, Pichler V, Mancini E, Pombi M, Vicente JL, Dinis J, Steen K, Petrarca V, Rodrigues A, Pinto J, Della Torre A, Weetman D. The last bastion? X chromosome genotyping of *Anopheles gambiae* species pair males from a hybrid zone reveals complex recombination within the major candidate “genomic island of speciation.” *Mol Ecol* 2016;25:5719–5731. <https://doi.org/10.1111/mec.13840>
- Charlwood JD, Jones MDR. Mating behaviour in the mosquito, *Anopheles gambiae* s. 1. save: I. Close range and contact behaviour. *Physiol Entomol*. 1979;4(2):111–120. <https://doi.org/10.1111/j.1365-3032.1979.tb00185.x>
- Charlwood JD, Jones MDR. Mating in the mosquito, *Anopheles gambiae* s.l. *Physiol Entomol*. 1980;5(4):315–320. <https://doi.org/10.1111/j.1365-3032.1980.tb00241.x>
- Charlwood JD, Pinto J, Sousa CA, Madsen H, Ferreira C, Do Rosario VE. The swarming and mating behaviour of *Anopheles gambiae* s.s. (Diptera: Culicidae) from São Tomé Island. *J Vector Ecol*. 2002;27(2):178–183.
- Coetzee M, Hunt RH, Wilkerson R, Torre AD, Coulbaly MB, Besansky NJ. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*. 2013;3619(3):246–274. <https://doi.org/10.11646/zootaxa.3619.3.2>
- Dabire KR, Sawadodgo S, Diabate A, Toe KH, Kengne P, Ouari A, Costantini C, Gouagna C, Simard F, Baldet T, et al. Assortative mating in mixed swarms of the mosquito *Anopheles gambiae* s.s. M and S molecular forms, in Burkina Faso, West Africa. *Med Vet Entomol*. 2013;27(3):298–312. <https://doi.org/10.1111/j.1365-2915.2012.01049.x>
- Dame DA, Curtis CF, Benedict MQ, Robinson AS, Knols BG. Historical applications of induced sterilisation in field populations of mosquitoes. *Malar J*. 2009;8(Suppl 2):S2. <https://doi.org/10.1186/1475-2875-8-S2-S2>
- Dao A, Adamou A, Yaro AS, Maiga HM, Kassogue Y, Traoré SF, Lehmann T. Assessment of alternative mating strategies in *Anopheles gambiae*: Does mating occur indoors? *J Med Entomol*. 2008;45(4):643–652. [https://doi.org/10.1603/0022-2585\(2008\)45\[643:aaamsi\]2.0.co;2](https://doi.org/10.1603/0022-2585(2008)45[643:aaamsi]2.0.co;2)
- Della Torre A, Tu Z, Petrarca V. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. *Insect Biochem Mol Biol*. 2005;35(7):755–769. <https://doi.org/10.1016/j.ibmb.2005.02.006>
- Diabate A, Baldet T, Brengues C, Kengne P, Dabire KR, Simard F, Chandre F, Hougaard JM, Hemingway J, Ouedraogo JB, et al. Natural swarming behaviour of the molecular M form of *Anopheles gambiae*. *Trans R Soc Trop Med Hyg*. 2003;97(6):713–716. [https://doi.org/10.1016/s0035-9203\(03\)80110-4](https://doi.org/10.1016/s0035-9203(03)80110-4)
- Diabaté A., Dabiré RK, Heidenberger K, Crawford J, Lamp WO, Culler LE, Lehmann T. Evidence for divergent selection between the molecular forms of *Anopheles gambiae*: role of predation. *BMC Evolution Biol*, 2008;8:1–11. <https://doi.org/10.1186/1471-2148-8-5>
- Diabate A, Dabire RK, Kengne P, Brengues C, Baldet T, Ouari A, Simard F, Lehmann T. Mixed swarms of the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in sympatric area from Burkina Faso *Journal of Med Entomol*. 2006;43(3):480–483.;
- Diabate A, Dabire RK, Kim EH, Dalton R, Millogo N, Baldet T, Simard F, Gimnig JE, Hawley WA, Lehmann T. Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: a transplantation experiment. *J Med Entomol*. 2005;42(4):548–553.
- Diabate A, Dao A, Diallo M, Huestis DL, Lehmann T. Spatial distribution and male mating success of *Anopheles gambiae* swarms. *BMC Evol Biol*. 2011;11:1–11.
- Diabate A, Dao A, Yaro AS, Adamou A, Gonzalez R, Manoukiss NC, Traoré SF, Gwadz RW, Lehmann T. Spatial swarm segregation and reproductive isolation between the molecular forms of *Anopheles gambiae*. *Proc R Soc B Biol Sci*. 2009;276(1676):4215–4222. <https://doi.org/10.1098/rspb.2009.1167>
- Diabate A, Tripet F. Targeting male mosquito mating behaviour for malaria control. *Parasit Vectors*. 2015;8(1):1–13.
- Diouf EH, Niang EHA, Samb B, Diagne CT, Diouf M, Konaté A, Dia I, Faye O, Konaté L. Multiple insecticide resistance target sites in adult field strains of *An. gambiae* (s.l.) from southeastern Senegal. *Parasit Vectors*. 2020;13:1–10. <https://doi.org/10.1186/s13071-020-04437-z>
- Fall PM. Evaluation de la sensibilité des vecteurs du paludisme aux insecticides dans la région de Kédougou et recherche de mécanismes de résistance; 2016. *Bibliothèque numérique, Université Cheikh Anta Diop*, 43p, <https://www.memoireonline.com>
- Gillies MT, De Meillon B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). 2d ed. *Johannesburg: South African Institute for Medical Research* 1968.
- Gimonneau G, Pombi M, Choisy M, Morand S, Dabiré RK, Simard F. Larval habitat segregation between the molecular forms of the mosquito *Anopheles gambiae* in a rice field area of Burkina Faso, West Africa. *Med Vet Entomol*. 2012;26(1):9–17. <https://doi.org/10.1111/j.1365-2915.2011.00957.x>
- Gueye OK, Tchouakui M, Dia AK, Faye MB, Ahmed AA, Wondji MJ, Nguiffo DN, Mugenzi LMJ, Tripet F, Konaté L, et al. Insecticide resistance profiling of *Anopheles coluzzii* and *Anopheles gambiae* populations in the Southern Senegal: role of target sites and metabolic resistance mechanisms. *Genes*. 2020;11:1403. <https://doi.org/10.3390/genes11121403>
- Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M. Transgenic Anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature*. 2002;417(6887):452–455. <https://doi.org/10.1038/417452a>
- Kamdem C, Tene Fossog B, Simard F, Etouana J, Ndo C, Kengne P, Boussès P, Etoa F-X, Awono-Ambene P, Fontenille D, et al. Anthropogenic habitat disturbance and ecological divergence between incipient species of the malaria mosquito *Anopheles gambiae*. *PLoS One*. 2012;7(6):e39453. <https://doi.org/10.1371/journal.pone.0039453>
- Killeen GF. A second chance to tackle African malaria vector mosquitoes that avoid houses and don't take drugs. *Am J Trop Med Hyg*. 2013;88(5):809–816. <https://doi.org/10.4269/ajtmh.13-0065>
- Lanzaro GC, Lee Y. Speciation in *Anopheles gambiae* – the distribution of genetic polymorphism and patterns of reproductive isolation among natural populations. In: Manguin S, editor. *Anopheles mosquitoes – new insights into malaria vectors*. InTech; 2013, pp. 173–196. <https://doi.org/10.5772/56232>
- Lehmann T, Diabate A. The molecular forms of *Anopheles gambiae*: a phenotypic perspective. *Infection, Genetics Evolut*, 2008;8(5):737–746. <https://doi.org/10.1016/j.meegid.2008.06.003>
- Marchand RP. Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. *Neth J Zool*. 1984;34(3):367–387. <https://doi.org/10.1163/002829684x00209>

- Moiroux N, Damien GB, Egrot M, Djenontin A, Chandre F, Corbel V, Killeen GF, Pennetier C. Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. *PLoS One*. 2014;9(8):e104967. <https://doi.org/10.1371/journal.pone.0104967>
- Moiroux N, Gomez MB, Pennetier C, Elanga E, Djènontin A, Chandre F, Djègbé I, Guis H, Corbel V. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *J Infect Dis*. 2012;206(10):1622–1629. <https://doi.org/10.1093/infdis/jis565>
- Moshi IR, Manderson L, Ngowo HS, Mlacha YP, Okumu FO, Mnyone LL. Outdoor malaria transmission risks and social life: a qualitative study in South-Eastern Tanzania. *Malar J*. 2018;17(1):397. <https://doi.org/10.1186/s12936-018-2550-8>
- Niang A, Epopa PS, Sawadogo SP, Maïga H, Konaté L, Faye O, Dabiré RK, Tripet F, Diabaté A. Does extreme asymmetric dominance promote hybridization between *Anopheles coluzzii* and *Anopheles gambiae* s.s. in seasonal malaria mosquito communities of West Africa? *Parasit Vectors*. 2015;8:586. <https://doi.org/10.1186/s13071-015-1190-x>
- Niang A, Sawadogo SP, Dabiré RK, Tripet F, Diabaté A. Assessment of the ecologically dependent post-zygotic isolation between *Anopheles coluzzii* and *Anopheles gambiae*. *PLoS One*. 2020;15(10):e0240625. <https://doi.org/10.1371/journal.pone.0240625>
- Niang EHA, Konaté L, Diallo M, Faye O, Dia I. Reproductive isolation among sympatric molecular forms of *An. gambiae* from Inland areas of South-Eastern Senegal. *PLoS One*. 2014;9(8):e104622. <https://doi.org/10.1371/journal.pone.0104622>
- Niang EHA, Konaté L, Diallo M, Faye O, Dia I. Patterns of insecticide resistance and knock down resistance (kdr) in malaria vectors *An. arabiensis*, *An. coluzzii* and *An. gambiae* from sympatric areas in Senegal. *Parasit Vectors*. 2016;9(1):1-7. <https://doi.org/10.1186/s13071-016-1354-3>
- Niang EHA, Konaté L, Faye O, Diallo M, Dia I. Vector bionomics and malaria transmission in an area of sympatry of *An. arabiensis*, *An. coluzzii* and *An. gambiae*. *Acta Trop*. 2018;189:129–136. <https://doi.org/10.1016/j.actatropica.2018.10.005>
- Nwakanma DC, Neafsey DE, Jawara M, Adiamoh M, Lund E, Rodrigues A, Loua KM, Konate L, Sy N, Dia I, et al. Breakdown in the process of incipient speciation in *Anopheles gambiae*. *Genetics*. 2013;193(4):1221–1231. <https://doi.org/10.1534/genetics.112.148718>
- Oliveira E, Salgueiro P, Palsson K, Vicente JL, Arez AP, Jaenson TG, Caccone A, Pinto J. High levels of hybridization between molecular forms of *Anopheles gambiae* from Guinea Bissau. *J Med Entomol*. 2008;45(6):1057–1063.
- Pates H, Curtis C. Mosquito behavior and vector control. *Annu Rev Entomol*. 2005;50:53–70. <https://doi.org/10.1146/annurev.ento.50.071803.130439>
- Poda SB, Nignan C, Gnankiné O, Dabiré RK, Diabaté A, Roux O. Sex aggregation and species segregation cues in swarming mosquitoes: role of ground visual markers. *Parasit Vectors*. 2019;12(1):1–15.
- Pombi M, Kengne P, Gimonneau G, Tene-Fossog B, Ayala D, Kamdem C, Santolamazza F, Guelbeogo WM, Sagnon N, Petrarca V, et al. Dissecting functional components of reproductive isolation among closely related sympatric species of the *Anopheles gambiae* complex. *Evol Appl*. 2017;10(10):1102–1120. <https://doi.org/10.1111/eva.12517>
- Ranson H, Lissenden N. Insecticide resistance in African anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol Special Issue Vectors*. 2016;32(3):187–196. <https://doi.org/10.1016/j.pt.2015.11.010>
- Sawadogo PS, Costantini C, Pennetier C, Diabaté A, Gibson G, Dabiré RK. Differences in timing of mating swarms in sympatric populations of *Anopheles coluzzii* and *Anopheles gambiae* s.s. (formerly *An. gambiae* M and S molecular forms) in Burkina Faso, West Africa. *Parasit Vectors*. 2013;6(1):117. <https://doi.org/10.1186/1756-3305-6-275>
- Sawadogo PS, Namountougou M, Toé KH, Rouamba J, Maïga H, Ouédraogo KR, Baldet T, Gouagna LC, Kengne P, Simard F, et al. Swarming behaviour in natural populations of *Anopheles gambiae* and *An. coluzzii*: review of 4 years survey in rural areas of sympatry, Burkina Faso (West Africa). *Acta Trop*. 2014;132(Suppl):S42–S52. <https://doi.org/10.1016/j.actatropica.2013.12.011>
- Sougoufara S, Diédhiou SM, Doucouré S, Diagne N, Sembène PM, Harry M, Trape J-F, Sokhna C, Ndiath MO. Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malar J*. 2014;13:1-7. <https://doi.org/10.1186/1475-2875-13-125>
- Sy O, Niang EHA, Ndiaye M, Konaté L, Diallo A, Ba ECC, Tairou F, Diouf E, Cissé B, Gaye O, et al. Entomological impact of indoor residual spraying with pirimiphos-methyl: a pilot study in an area of low malaria transmission in Senegal. *Malar J*. 2018;17(1):1-11. <https://doi.org/10.1186/s12936-018-2212-x>
- Torre A, Fanello C, Akogbeto M, Dossou-yovo J, Favia G, Petrarca V, Coluzzi M. Molecular evidence of incipient speciation within *Anopheles gambiae* s.s. in West Africa. *Insect Mol Biol*. 2001;10(1):9–18. <https://doi.org/10.1046/j.1365-2583.2001.00235.x>
- Touré YT, Petrarca V, Traoré SF, Coulibaly A, Maïga HM, Sankaré O, Sow M, Di MD, Coluzzi M. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia*. 1998;40:477–511.
- Tripet F, Touré YT, Taylor CE, Norris DE, Dolo G, Lanzaro GC. DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae*. *Mol Ecol*. 2001;10(7):1725–1732. <https://doi.org/10.1046/j.0962-1083.2001.01301.x>
- Wang G, Vega-Rodríguez J, Diabate A, Liu J, Cui C, Nignan C, Dong L, Li F, Ouedraogo CO, Bandaogo AM, et al. Clock genes and environmental cues coordinate *Anopheles* pheromone synthesis, swarming, and mating. *Science*. 2021;371(6527):411–415. <https://doi.org/10.1126/science.abd4359>
- WHO. *World malaria report 2021*. Geneva: World Health Organization; 2021. <https://apps.who.int/iris/handle/10665/350147>.
- Wilkins EE, Howell PI, Benedict MQ. IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to diel-drin in *Anopheles arabiensis*. *Malar J*. 2006;5(1):1-7. <https://doi.org/10.1186/1475-2875-5-125>
- Yuval B. Mating systems of blood-feeding flies. *Annu Rev Entomol*. 2006;51:413–440. <https://doi.org/10.1146/annurev.ento.51.110104.151058>
- White BJ, Kundert PN, Turissini DA, Van Ekeris L, Linsler PJ, Besansky NJ. Dose and developmental responses of *Anopheles merus* larvae to salinity. *J Exp Biol*. 2013;216(Pt 18):3433–3441. <https://doi.org/10.1242/jeb.087189>