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Dynamics of transmission of *Plasmodium falciparum* by *Anopheles arabiensis* and the molecular forms M and S of *Anopheles gambiae* in Dielmo, Senegal

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Background

The *Anopheles gambiae* complex consists of at least seven species among which *Anopheles gambiae* s.s. is one of the most anthropophilic malaria vectors in Africa [1]. The adaptation of *An. gambiae* to humans and its environment involves an ongoing speciation process that can be best demonstrated by the existence of a number of incipient taxonomic units, characterized by the presence of paracentric inversions leading to different chromosomal arrangements [2]. This speciation process is primarily observed in West Africa, where five chromosomal forms of *An. gambiae* s.s. have been described and designated with a non-Linnean nomenclature: *bamako*, *bissau*, *forest*, *mopti* and *savanna* [3,4].

During the last few years, several research teams have settled on a molecular approach to address speciation in *An. gambiae* s.s.. Various degrees of gene flow restriction were demonstrated between chromosomal forms, with strong hybrid heterokaryotype deficits in the areas of sympatry. Analysis of the rDNA intergenic spacers, located on the X-chromosome, revealed fixed sequence differences between sympatric and synchronous *savanna*/*bamako* and *mopti* populations in Mali, Burkina Faso and Cameroon [2,5,6]. To provide more insight into their taxonomic status, recent efforts have focused on the pattern of variation observed with molecular markers. This revealed the existence of two genetic variants referred to as the molecular M and S forms [7,8].

However, whatever the geographical region, it has been clearly demonstrated that the gene flow between M and S forms is very limited, revealing a current speciation phenomenon. The genetic characteristics of these forms and their known geographical distribution have recently been reviewed [9]. Studies carried out so far have shown that the M and S forms may have different habitat even in sympatric areas [5,10].

The aim of this study was to compare the dynamics of transmission of *Anopheles arabiensis* and *An. gambiae* M and S molecular forms in a Senegalese village, where the two forms coexist.

Methods

Study area

The village of Dielmo (13°45N, 16°25W) is located in an area of Sudan-type savanna, 280 km Southeast of Dakar and about 15 km north of the Gambian border. About 300 inhabitants are living in the village. Rainfall occurs during a four-month period, from June to October. The average annual rainfall during our study period in 2004 was 642.4 mm. Dielmo is situated on the marshy bank of a small permanent stream, the Nema, where anopheline larval sites are present all year round. Only few cattle are living in this area [11].

Mosquito collections

Adult mosquitoes were collected monthly from July to December 2004. Two sampling methods were used: night landing catches (NLC) and pyrethrum spray catches (PSC). Hourly NLC were made on edult ,e aim of stl yrfews to s to d p t-5.6(tosmall) collectober. athrrestrea catc8.The

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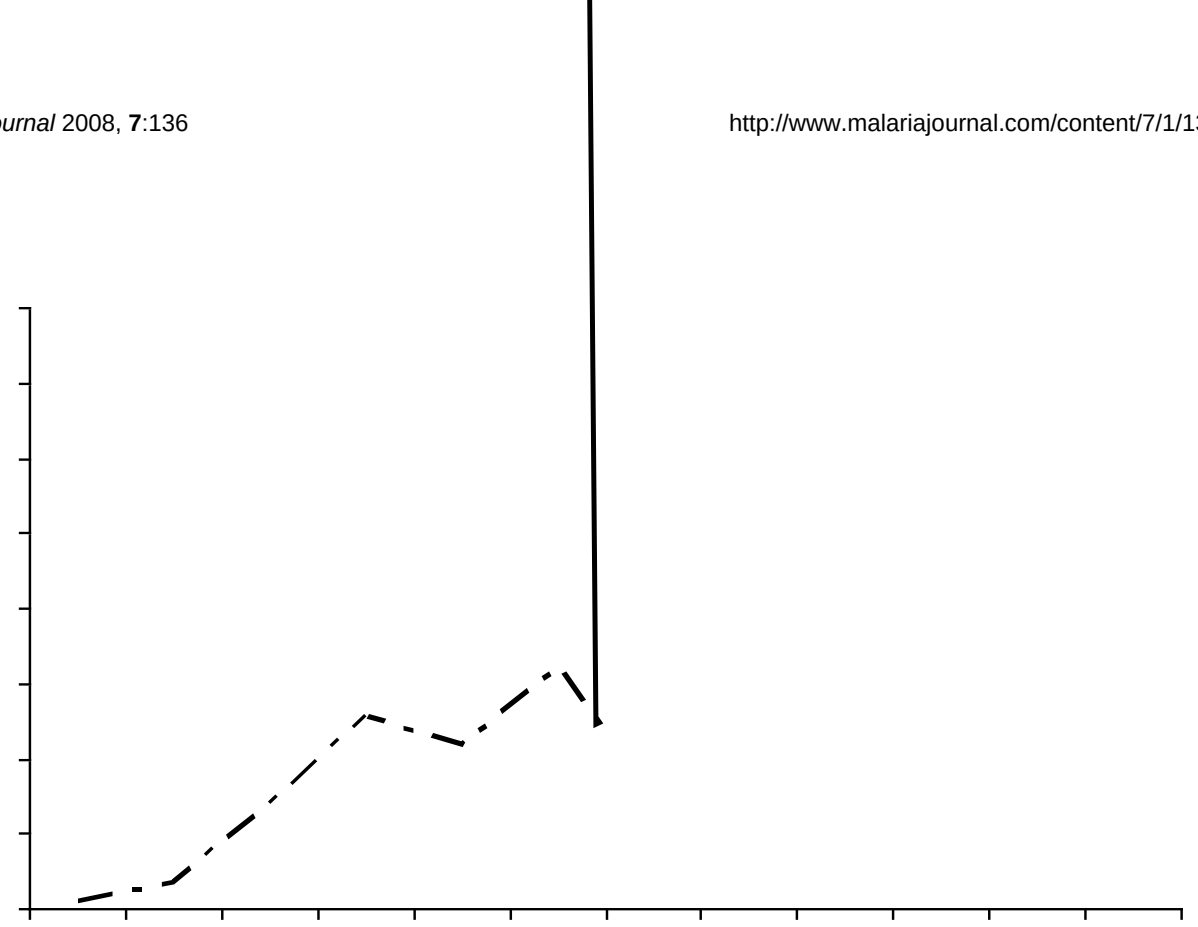

Figure 1
The nocturnal biting cycle of *An. arabiensis* and *An. gambiae* M and S molecular forms, from July to December 2004, in Dielmo, Senegal.

which are greater than those previously recorded in other parts of Senegal [1,18,20,21]. The high CSP rate in *An. arabiensis* is probably explained by the high antropophilic behaviour in this particular setting. Highton *et al* [22] and Joshi *et al* [23] in the same region Kisumu, found high and similar CSP rate for *An. gambiae* s.s. (5.3% and 7.5% respectively), while the CSP rate was very different for *An. arabiensis* (0.3% and 7.5% respectively). The difference observed in *An.arabiensis* was due to cattle abundance and movements.

A recent analysis of published and unpublished data on the molecular forms of *An. gambiae* has demonstrated that the M form shows a more latitudinal range in West Africa than the S form, being the only form recorded in the Sahelien region of northern Senegal [9,24]. In Dielmo, the M form was mainly observed in rainy season from August to October, with a maximum in September. The S form was observed in the rainy season as well, with a maximum in August, but was also found during the dry season. *An. arabiensis* had an approximately constant density during the rainy season and the beginning of the dry season, without an obvious peak. Thus rainfall alone can not explain fluctuations observed in M and S forms densities.

Cytogenetic studies were conducted in 1991 in Dielmo and revealed an important chromosomic polymorphism. Thirty-two half-gravid females collected in January and February 1991 showed chromosomal inversions characteristic of *bissau* chromosomal form which belongs to the M molecular form [8]. Twenty-four females collected in September 1991 mainly revealed *savanna* cytotype which belongs either to M form or S form [8]. In this study, the replacement of S molecular form by M molecular form in the late rainy season might reflect the replacement of *savanna* chromosomal form by *bissau* chromosomal form. Climate (rainfall, temperature, relative humidity) as well as nature of anopheline larval development sites are dramatically change throughout the year during the rainy season. As chromosomal inversions are known to be involved in adaptation to climate and environment [1], the correspondence of molecular and chromosomal forms may explain the distribution pattern of molecular forms.

In West Africa, there is evidence of varying levels of hybridation between M and S forms, a mechanism by which adaptive genes may flow from one to the other, including those conferring insecticide resistance [25,26]. No, or very

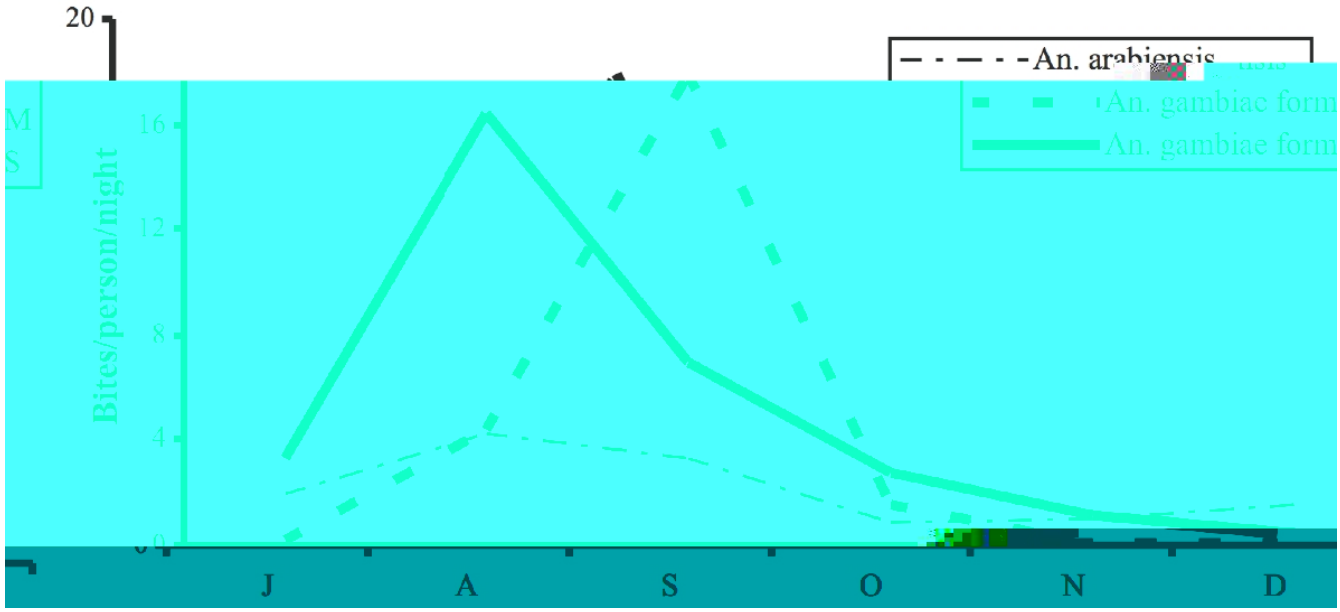

Figure 2
Monthly human biting rates of *An. arabiensis* and *An. gambiae* M and S molecular forms, from July to December 2004, in Dielmo, Senegal.

few, M/S hybrids were observed throughout Cameroon [6,27], Ghana [10], Mali and Burkina Faso [9]. In this study, 22 M/S hybrids were observed (3.05%). Despite this number of hybrids, the *An. gambiae* population is far from Hardy Weinberg equilibrium suggesting restricted gene flow between M and S form. The occurrence of M/S hybrids in the field, however, has been reported several times. Della Torre *et al* [8] found three hybrids patterns out of 1,161 *An. gambiae* adult mosquitoes tested from throughout Africa (hybrid frequency = 0.26%). The authors, therefore, mentioned contamination as a possible cause of the hybrid patterns they reported. Further evidence for the viability of M/S hybrids in the wild was provided by Taylor *et al* [28], who reported occurrence of M/S hybrid larvae at a frequency of 0–1.29% in Banambani (Mali). Tripet *et al* [29] identified an inseminated female showing the M/S pattern, which demonstrated that M/S hybrids could be produced in the field, survive up to the adult stage and are reproductively active.

Conclusion

Anopheles arabiensis and the M and S molecular forms of *An. gambiae* coexist in Dielmo village. No difference was observed either in host preference or in *Plasmodium falciparum* infection rates between sympatric M and S populations, but they present different dynamics of transmission: the S form is the major vector during the first part of the rainy season and is replaced by M form later in the season. These variations are probably attributable to different breeding conditions.

Table 2: Infection rate for *P. falciparum* calculated by circumsporozoite protein (CSP) ELISA from the head and thoraxes of *An. arabiensis*, *An. gambiae* M and S forms in Dielmo

Month	*An. arabiensis*		*An. gambiae* M Form		*An. gambiae* S Form	
	Tested	Positive	Tested	Positive	Tested	Positive
July	27	2	4	1	49	1
August	56	1	61	2	211	5
September	49	0	225	4	91	5
October	12	1	22	3	34	2
November	13	1	2	0	14	1
December	20	0	1	0	6	0
CSP rates [95% cl]	2.82% [0.9–6.5]		3.17% [1.5–5.8]		3.45% [1.9–5.7]	

Authors' contributions

MON and DF have equally contributed to the design, acquisition, analysis, interpretation of data and manuscript drafting. LK contributed to conception of study and contributed markedly to the analysis of entomological data. CB for field activities and Molecular biology. JFT and CB participated in the conception and coordination of the study and helped to draft the manuscript. CS provided the scientific supervision in Dielmo.

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