

Natural swarming behaviour in the molecular M form of *Anopheles gambiae*

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Abstract

In *Anopheles gambiae*, as in most species of mosquitoes, mating is initiated in flight. The males aggregate in aerial swarms and conspecific females individually fly to these swarms where they mate with males. In this study, we investigated the swarming behaviour of *A. gambiae* and conducted 2 surveys in the rice field area of the Vallée du Kou in Burkina Faso in 1999 and 2002. A high number of anopheline mosquitoes were observed in this area and both molecular M and S forms of *A. gambiae* were found in sympatry. Swarms formed a few minutes after sunset in different places and no obvious markers were associated with their occurrence. However, swarms occurred close to cow herds generally in open flat areas, 2–3 m above the ground. Overall, 2823 anopheline mosquitoes were collected from 21 swarms composed primarily of males. A few specimens of *Culex quinquefasciatus* were collected from 3 swarms. Although both molecular M and S forms were found in sympatry in the village, swarms were composed almost exclusively of the molecular M form. This suggests that there are alternative swarming habits for both M and S molecular forms of *A. gambiae* in nature.

Keywords: *Anopheles gambiae*, molecular forms, mating behaviour, Burkina Faso

Introduction

Despite decades of research, *Anopheles gambiae sensu stricto*, the major malaria vector in sub-Saharan Africa, continues to reveal novel complexities. Originally considered as a single species, evidence for genetic heterogeneity within *A. gambiae* began to accumulate in the early 1980s (Coluzzi *et al.*, 1985; Touré *et al.*, 1994, 1998). A molecular test undertaken on a number of *A. gambiae* populations in West Africa has recently split this species into 2 molecular forms, termed M and S, which are assumed to be reproductively isolated (della Torre *et al.*, 2001; Favia *et al.*, 2001). Many laboratory and field studies have investigated reproductive isolation and speciation between these 2 molecular forms with conflicting results (Chandre *et al.*, 1999; Weill *et al.*, 2000; della Torre *et al.*, 2001; Gentile *et al.*, 2001; Tripet *et al.*, 2001; Diabaté *et al.*, 2002, 2003; Wondji *et al.*, 2002). It is unclear whether these forms can be considered as 'true' species (Black & Lanzaro, 2001; della Torre *et al.*, 2001; Gentile *et al.*, 2001). Although the molecular forms display no post-mating reproductive isolation in the laboratory (Chandre *et al.*, 1999), the hybrid individuals are extremely rare in nature and assortative mating within both molecular forms has been demonstrated (Tripet *et al.*, 2001). This implies that the genetic isolation mechanism is probably acting at pre-mating level (Touré *et al.*, 1998; Gentile *et al.*, 2001).

Diptera differ from most insect groups in their ability to mate in flight (Downes, 1991). Mating systems based on aerial male aggregations that function as encounter sites for mate-searching females have evolved repeatedly in various groups of insects (Sullivan, 1981; Cooter, 1989). In most swarming species, the swarms are composed of males; females typically approach a swarm, acquire a mate, and leave *in copula*. Insects use a variety of stimuli to bring males and females together for mating, of these, volatile sex pheromones and sound can function over substantial distances (Clements, 1999). The way the sexes are attracted may contribute to specific mate recognition

systems, which facilitate species identification and prevent hybridization. The different methods of assembly not only bring conspecific males and females together but reduce the risk of contact between males and females of different species (Clements, 1999). If a genetic isolation mechanism is acting at a pre-mating level between the M and S forms of *A. gambiae* it should be observable through careful study of mating behaviour in areas where both forms occurs in sympatry. How and where do the molecular M and S forms of *A. gambiae* mate? Do they co-swarm or not? This paper gives preliminary results of a study of swarming behaviour in *A. gambiae*, defining the time and place at which males and virgin females are brought into close proximity.

Materials and Methods

Study area

Two surveys were conducted in 1999 and in 2002 in the Vallée du Kou of Burkina Faso, a rice cultivation area developed in 1970. The area is characterized by wooded savannah and covers 1200 ha between 4°24' W and 11°24' N and contains 7 discrete villages. Mean annual rainfall is about 1200 mm and rice is the major crop. Two seasons characterize the area, a rainy season in May–October and a dry season in November–April. The river Kou is a permanent source of irrigation and there are 2 rice crops per year (July–November and January–May). Because of the irrigation system, the rice fields form permanent mosquito breeding sites which are preferentially colonized by the Mopti chromosomal form. The Mopti form corresponds to the molecular M form in Burkina Faso (della Torre *et al.*, 2001). During the rainy season, additional breeding sites are found in depressions and ponds. The village has more than 600 inhabitants who are mainly farmers. Sheep, goats, pigs and cows are present.

Mosquito collection and analysis

Adult female night catches and observations of swarming were performed in VK7, a village located on the boundary of the rice and cotton areas. Adult females were regularly collected by human bait catches from July to December 1999 to estimate population dynamics of *A. gambiae* in the village with special interest on temporal variation in the relative frequencies of both molecular forms. Swarms were analysed

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monthly from July to December 2002. Observations were made on swarms which occurred after sunset in places bordering the rice field breeding sites, then in places inside the village distant from the rice fields. Previous studies showed a relatively high frequency of the Savannah chromosomal form in these places distant from the rice fields (Robert *et al.*, 1989). This chromosomal form corresponds to the molecular S form in Burkina Faso (della Torre *et al.*, 2001). After observation for several minutes, swarms were sampled with an insect net. Mosquitoes were aspirated into cups, killed with chloroform, identified, counted and placed on silica gel in tubes. Genomic DNA was subsequently extracted from single field-collected mosquitoes and amplified by polymerase chain reaction (PCR) to determine the molecular (M or S) form within *A. gambiae* (Favia *et al.*, 2001).

Results

Monthly variation in *Anopheles gambiae* density

The number and relative proportion of each molecular form of *A. gambiae* varied throughout the year. Considerable numbers of mosquitoes were collected from July to October with a peak of abundance reaching 200 bites/person/night in July, when the rice fields offered many breeding sites (Figure). PCR analysis showed that both M and S molecular forms were found in sympatry in the study area during this period (Figure). Overall, 236 mosquitoes were analysed from July to December 1999. The molecular M form was predominant throughout the season. At least 30 individuals were analysed per month. The molecular S form was absent in July. This form appeared in August and increased in frequency throughout the season, reaching a peak of 38% in November (Figure). Similar results were obtained in 2000.

Observations of swarms

Swarms were observed in 10 sites spread throughout the village. As previous studies showed a high relative frequency of the molecular S form in sites distant from the rice fields, efforts were made to localize swarms in these parts of the village. Swarms were sampled in 7 sites inside the village which were distant from the rice fields and in 3 sites at the edge of the rice fields. In all sites, swarming generally began 5–10 min after sunset and stopped with the onset of darkness 20–30 min later. Swarms were always initiated by a small number

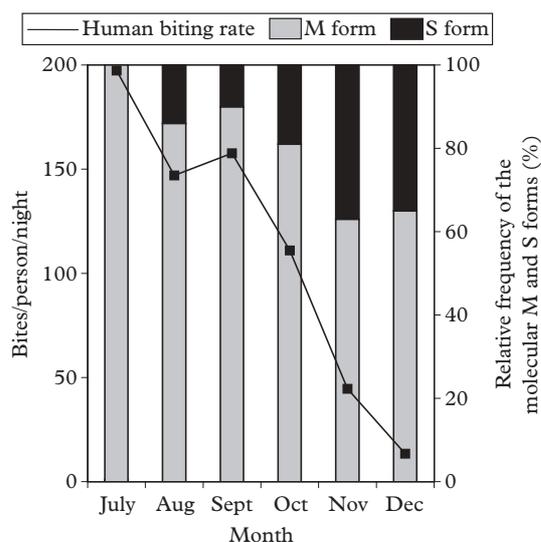


Figure. Monthly variation in *Anopheles gambiae* density and the relative frequency of the molecular M and S forms in VK7 village of the Vallée du Kou, Burkina Faso in 1999.

of males, dancing in flight, that were progressively joined by other males until the swarm density reached several hundred. After the swarm had formed, mosquitoes flew in tall vertical and shorter horizontal loops occasionally moving forwards, backwards or up and down around a pivotal position. No discernible marker was associated with the swarm formation. In the 10 swarming sites, 5 were in open flat areas, 2 were above wells, 1 was above a hen house and 2 above dumps. Once a swarm was localized, it reformed daily with some exceptions throughout the season at approximately the same place. Swarms were often localized approximately 2–3 m above the ground. Eight of the 10 swarm sites occurred close to cow herds. The farthest one was located 10 m from the cow herd.

Specific composition of swarms

On 12 evenings of observation, 2823 mosquitoes were collected from 21 swarms (Table). Swarms were almost exclusively composed of males. Six females were caught in August and September. Overall, 1256 anopheline mosquitoes were sampled from 9 swarms in the village bordering the rice fields whereas 1567 were sampled from 12 swarms inside the village. We genotyped 269 males and 6 *A. gambiae* females to determine the specific composition of swarms collected throughout the season. Although both molecular M and S forms were found in sympatry in the village, swarms were composed exclusively from the M form. Special efforts were made to sample swarms in sites distant from the rice fields where the molecular S form was relatively abundant but the S form was not caught in July nor in October–December when the relative frequency of this form increased (Figure). In contrast, 7 female *Culex quinquefasciatus* were caught from 3 swarms in August and September.

Discussion

Aggregation is an important first phase in the mating behaviour of most mosquitoes (Clements, 1999). Swarming in *A. gambiae* is the time at which insemination predominantly occurs (Charlwood & Jones, 1979). In Burkina Faso, swarms were observed about 5–10 min after sunset. They were always initiated by a few males dancing in flight, which were progressively joined by other males until the number reached several hundred. The aggregations progressively dispersed as the light faded, 20–30 min after forming. Swarms were not over sites with obviously similar characteristics but most of them were localized close to cow herds. However, cows may not be essential determinants for swarm formation. In Tanzania, Marchand (1984) observed no discernible markers associated with swarms of *A. gambiae* and *A. arabiensis* which occurred above flat, open ground within an area of scattered huts and trees. However, these mosquitoes use horizon markers for orientation and require a minimum angle of view of the sky. In the Gambia, 2 factors appeared to be important in determining the arena of anopheline: first, its openness and second, features associated with the surrounding skyline (Charlwood & Jones, 1980). Few *A. gambiae* females were collected from swarms, which raises the question of the reproductive function of the crepuscular swarms formed by most mosquito species (Nielsen & Heager, 1960; Downes, 1969). Most authors agree that while swarms are composed primarily of males, females approach these and are promptly mated (Quarishi, 1965; Reisen & Aslamakhan, 1976; Reisen *et al.*, 1977; Marchand, 1984; Yuval *et al.*, 1992). Males swarm every day of their post-teneral life while females generally mate only once and change their behaviour after insemination. Thus, assuming that the gender ratio is equal, one would expect a low rate of copulation in swarms (Nielsen & Heager, 1960; Jones & Gubbins, 1978; Charlwood & Jones, 1980). All *A. gambiae* swarms sampled throughout the season

Table. Number of mosquitoes collected and specific composition of swarms from July to December 2002 in VK7 village of the Vallée du Kou, Burkina Faso

	No. of mosquitoes sampled from swarms		Molecular forms of <i>Anopheles gambiae</i> analysed from swarms ^a	
	Males	Females	M form	S form
July	164	0	100% (60)	0% (0)
August	757	3	100% (60)	0% (0)
September	1158	3	100% (60)	0% (0)
October	66	0	100% (35)	0% (0)
November ^b	—	—	—	—
December	678	0	100% (60)	0% (0)

^aRelative frequency of the molecular forms of *Anopheles gambiae* (no. of mosquitoes analysed).

^bObservation not done in November.

were composed of the molecular M form. As the M and S forms are in sympatry in this study area it is surprising that no S forms were recorded. This raises the question of whether the swarming conditions of the molecular M and S forms of *A. gambiae* differ. Most investigators have found swarms to be composed of males of a single species, even when several species were swarming in the same area (Clements, 1999). However, mixed swarms have occasionally been reported. *Anopheles funestus* swarmed at sites only marginally different from those used by sympatric populations of *A. gambiae* and *A. arabiensis* and overlapped with them to such an extent that mixed swarms were common (Marchand, 1984). A few female *C. quinquefasciatus* were occasionally recorded in 3 of the 21 swarms observed in our study. Mosquito species which have the same characteristics of swarming may form mixed swarms in sympatric areas but this does not mean that they will copulate. Males and females must be close before mating. Swarming males erect their antennal fibrillae probably to identify and locate conspecific females that approach the swarm by their flight tone (Clements, 1999). Most experimental and observational evidence suggests that male mosquitoes respond to female flight tone over distances of 5–10 cm. Caged *A. gambiae* males responded to tones from a 2 mm diameter artificial sound source responding most strongly to emission of 450–500 Hz and orientating towards the source with great accuracy (Clements, 1999).

Studying the sexual behaviour of the molecular M and S forms of *A. gambiae* is important in order to investigate incipient speciation issue within this taxon. From this study, it is difficult to make definitive conclusions, but because no molecular S forms were recorded from 21 swarms it is probable that the 2 molecular forms have different swarming characteristics. This suggests alternative swarming habits for both the molecular M and S forms of *A. gambiae* in this area of sympatry, a putative pre-mating isolation mechanism that may concur to the systematic deficit in M/S hybrids generally observed in the field, despite no evidence for post-mating isolation between forms. Further studies of swarming may help to understand the phenomena.

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