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Observations on the swarming and mating behaviour of *Anopheles funestus* from southern Mozambique

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Abstract

Background: Control of malaria by the release of genetically modified mosquitoes refractory to transmission is now becoming a possibility. In many areas of Africa, *Anopheles gambiae* is found together with an equally important vector, *An. funestus*. Given their sympatry and the likelihood of a similar mating period some aspects of the mating behaviour of *An. gambiae* s.l. and *An. funestus* are likely to differ. We therefore attempted to characterise the swarming behaviour of *An. funestus* and to determine if any aspects of the observed behaviour differed from that recorded for the M form of *An. gambiae* from São Tomé.

Methods: In March – May 2002 the swarming, mating, house exiting and resting behaviour of *Anopheles funestus* was studied by direct observation in Mozambique. Swarming males and insects in copula were collected by sweep net. Wing lengths of males collected resting, exiting houses, swarming and mating were measured and the wingbeat frequency distribution of individual insects, in free flight confined inside netting covered paper cups, was also determined.

Results: Mono-specific swarms occurred at sunset in relatively open areas close to houses used for resting. Mating pairs were seen 11 ± 3.7 min after the start of swarming. The number of total pairs observed being inversely proportional to the time difference between the start of swarming and the first pairing. The great majority of females mated before feeding. Male or female size did not appear to affect mating success or other behaviours. During the study, ambient temperatures decreased and female, but not male, wing size increased. At 516 Hz, the flight tone of female *An. funestus* was similar to the 497 Hz of the local *An. gambiae*. Males dispersed if light or dark artificial horizontal markers were placed underneath naturally occurring swarms.

Conclusion: Differential response to markers would be sufficient for swarming in *An. funestus* and *An. gambiae* s.l. to occur in distinct sites.

Background

Control of malaria by the release of genetically modified mosquitoes refractory to transmission is now becoming a possibility. Should the 'leap of faith' ever be taken it is likely that releases will consist only of males [1]. It has been estimated that introduction will succeed only if fit-

ness is greater than 80% [2]. The more fitness in released males can be enhanced, the better.

One of the prime targets for such modification is *Anopheles gambiae*, one of the primary vectors in sub-Saharan Africa, where more than 90% of the world's malaria is

transmitted. Whilst size of males does not appear to influence mating success [3] age might. Females that mate with males that are two days old may be more likely to oviposit than if they mate with older males [4]. Hence, younger males may be fitter and, for the introduction to succeed, released males will need to mate with wild females early in their life.

In many areas of Africa *Anopheles gambiae* is found together with an equally important vector, *An. funestus*. Eliminating transmission by *An. gambiae* without affecting transmission by *An. funestus* is likely to have little long-term effect on malaria in many areas.

Given their sympatry and the likelihood of a similar mating period, some aspects of the mating behaviour of *An. gambiae* and *An. funestus* are likely to differ. These presumably involve a spatial element and, in *An. gambiae*, like many other anophelines, mating is associated with swarming [5–9]. The only known record of swarming in *An. funestus*, however, is that of Harper [10] who observed swarming males on the threshold of a thatched dwelling 1 km from Nyanza, Lake Victoria [10]. Indeed the doyens of African medical entomologists, Gilles and de Meillon, in their extensive review [11] state, 'We have never found them (swarms) ourselves, even in areas of high density'.

An. gambiae swarm in relatively open areas over horizontal areas of contrast in the environment [5]. The greater the contrast the more likely will a swarm form and they can be induced to form over artificial markers. Although thought to be a eurygamic species (which requires large volumes of space to successfully mate) Harper [10] describes *An. funestus* as 'dancing up and down about a foot inside the room'. No marker was described and no mating was recorded despite the fact that estimated numbers in the swarm, which stretched almost a metre across, exceeded 500.

In a recent study of swarming behaviour of *An. gambiae* in São Tomé Charlwood *et al.*, [5], however, considered that swarm site and marker were unlikely to be specific to a particular species. They also threw doubt on the idea of 'swarm arenas' proposed by Charlwood and Jones [12]. Swarms of *An. funestus* were commonly observed, in a village in southern Mozambique, in areas similar to those used by *An. gambiae* in São Tomé.

We therefore attempted to characterise the swarming behaviour of *An. funestus* in these areas and to determine if any aspects of the observed behaviour differed from that recorded for the M form of *An. gambiae* from São Tomé [5].

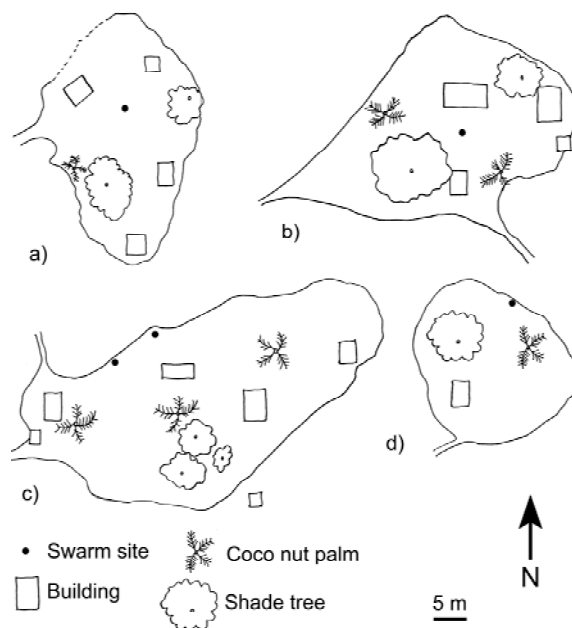


Figure 1
Map of four sites in which swarms of *Anopheles funestus* were observed, Furvela village, Mozambique.

Methods

The study took place on nineteen evenings between the 23rd March and 3rd May 2002, in the village of Furvela, 650 km north of Maputo and 7 km south of the town of Morrumbene, in the province of Inhambane, on the main Maputo-Beira highway (EN1). The village lies circa 3 km from mangrove-bordered coast and is delineated by the Furvela river a small stream within a 2 km wide valley. Most of the villagers live by subsistence farming, growing maize, manioc, peanuts and beans. Cashew nut trees and coconut palm are common. In the river valley sugar cane, dry rice and bananas are grown. *An. funestus* is the most abundant mosquito in the village although members of the *An. gambiae* complex, *An. tenebrosus*, *Mansonia* spp. and a variety of culicines are also common.

Direct observation of swarms was undertaken in the manner described by Charlwood & Jones [12] and Charlwood *et al.*, [5]. Swarming males and insects in copula were collected by sweep net, modified in the manner described by Marchand [7]. Artificial markers used were the same as those used in São Tomé [5].

In order to determine whether size affected likelihood of a male swarming or mating the wing lengths of males



Figure 2
Swarm site b) looking to the west. The arrow indicates the approximate location of the swarm.

collected resting, exiting houses, swarming and mating were measured. Collections of indoor resting insects were performed, with a torch and aspirator, for 10 minutes in buildings shown in figure 1 and exiting insects at dusk were monitored by placing a conical, double-size bednet over the gable-ends of houses within the study area.

Wing lengths, between the alula notch and the wing tip, excluding scales, of unselected samples of insects from all collections were measured using an ocular micrometer on a stereoscope to the nearest 0.03 mm. Wing lengths of the different groups were compared using ANOVA.

In order to determine if differences in flight sound between *An. funestus* and *An. gambiae*, which might swarm together, differ (and therefore may act as potential barrier to prevent hybridization) the frequency distribution of individual insects, in free flight confined inside netting covered paper cups, was determined according to the method outlined in Brogdon [13,14]. Briefly, recordings of individual insects were made with a microphone (a Sony ECM-D870P) either directly onto a portable computer or onto mini-disc and then transferred to computer and stored as wave files. The files were digitally sampled at 20,000 Hz and then re-sampled at 5,000 Hz and sonograms, plots of sound frequency versus time, prepared using computer software (Spectrogram, public domain by Richard Horne <http://www.visualizationsoftware.com/gram/gramdl.html>). All recordings were made on the same afternoon at the same temperature. The flight tone

of both male and unfed females (of both *An. funestus* and *An. gambiae* s.l.) was determined. Temperature data was obtained for the two nearest weather stations (Inhambane and Vilanculos) available from the NOAA website <ftp://ftp.ncdc.noaa.gov/pub/data/global sod/> and the mean value used.

Results

Swarms of male *An. funestus* were observed within the sandy clearings surrounding houses. Swarms formed in the same place on successive evenings. There was, however, no obvious pattern as to the location of the swarms which could be at the edge or in the middle of the clearing (Figure 1, Figure 2). Swarms occurred 2–4 m off the ground, occupied a similar volume, and appeared to consist of a similar number of insects. At some sites two or more swarms occurred within a few metres of each other (Figure 1). It was not possible to determine with any certainty, what the characteristics were that enabled males to maintain their position. At the site closest to the river valley (the putative breeding site) the swarm occurred in a gap in vegetation that was illuminated by the western sky (Figure 1a). When this gap was artificially closed (by extending a sheet across it) males flew higher (so that they continued swarming in a gap). Two further swarms were discovered by looking for such gaps, and similar profiles occurred at other swarm sites, although in all other cases the 'silhouette' was created at a much greater distance from the place where the swarm occurred.

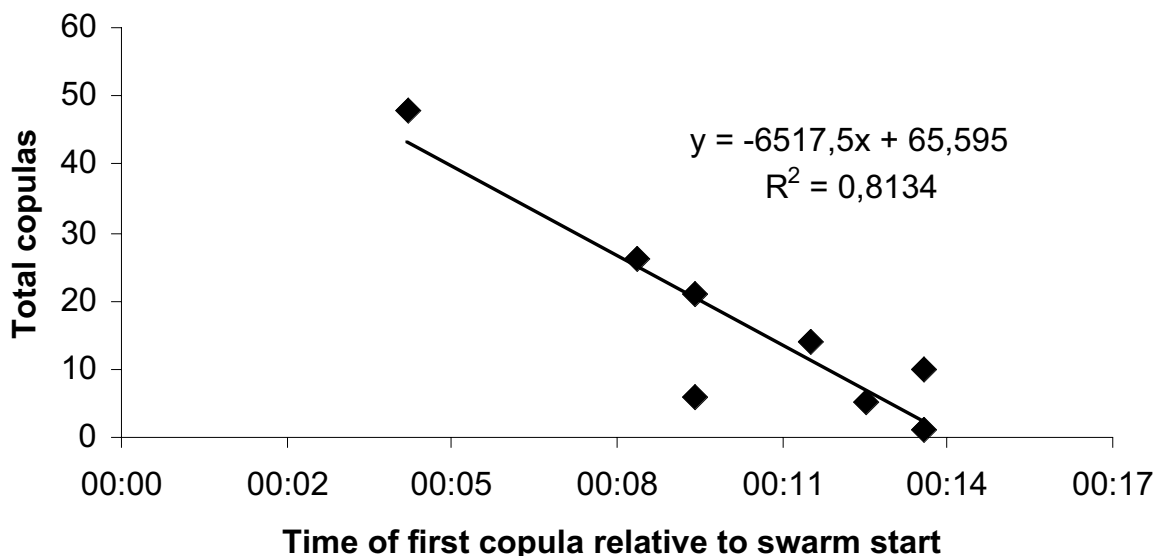


Figure 3

Relationship between the time of the first copula observed after the start of swarming and the total number of pairs subsequently counted.

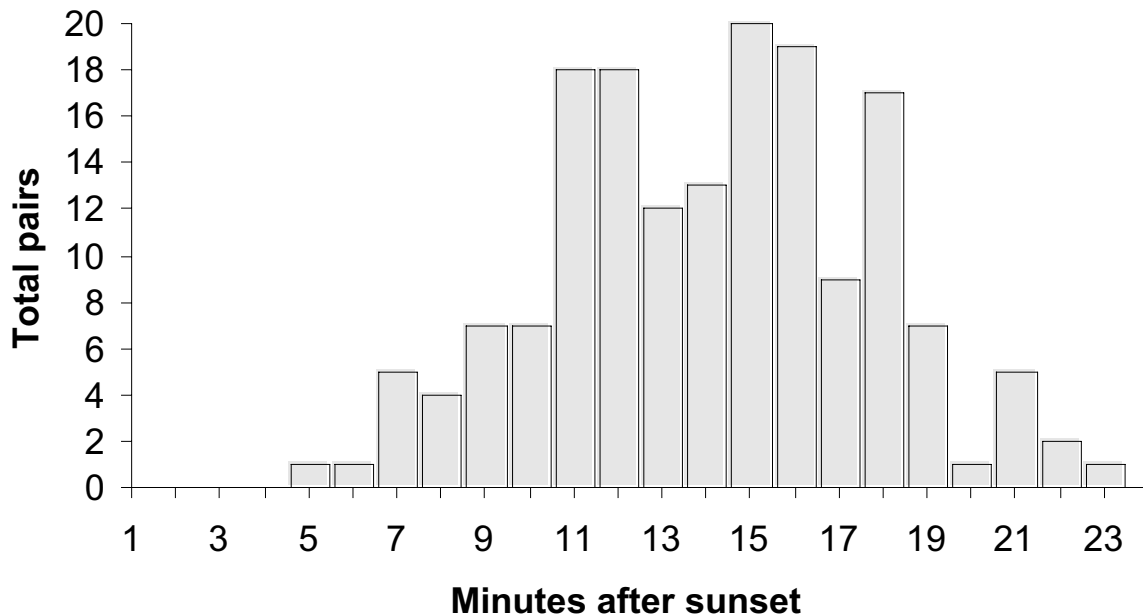
Swarming was co-incident with males leaving houses at sunset. It was rarely possible to see the first male at the swarm site; rather, several insects started swarming at the same time. Numbers rapidly built up and within five minutes of the start had reached maximum size and density. On two occasions, dragonflies (*Libellula* sp.) were seen to capture and eat males at the start of the swarming period. On one occasion all four males in a swarm were caught and eaten in a matter of seconds.

On one occasion 1 *An. gambiae* s.l. and 3 *An. funestus* were collected from a swarm of apparently larger insects that formed close to a higher and larger swarm from which 1 *An. gambiae* s.l. and 32 *An. funestus* were subsequently collected. On all other occasions only male *An. funestus* were collected swarming (a total of 601 males from 13 swarms)

In sharp contrast to the *An. gambiae* from São Tomé [5], swarming males avoided black or white horizontal markers and dispersed when these were introduced under the swarm. Despite there being a considerable number of male *An. gambiae* s.l. resting inside houses, swarms of this species were not induced by the presence of artificial markers.

The number and time of pairs in copula was noted on 9 evenings of observation. An average of 16 (s.d. 14.3, range 1–48) pairs in copula were seen. The time at which the first pair was observed, relative to the time of the start of swarming, was inversely proportional to the total number observed throughout the swarming period (Figure 3) Limitation of resources meant that comparisons between sites on the same day were unavailable. Mating pairs were first seen 11 +/- 3.7 min after the start of swarming and had reached a maximum 4 min later. By the end of the observation period (when it became impossible to see the insects with the naked eye) numbers of mating pairs had almost declined to zero (Figure 4).

Female *An. funestus* rarely took a blood meal before mating. Thirty-one of the 32 females collected in copula were unfed. The remaining female had taken a small blood meal prior to mating. All were newly emerged with undeveloped ovaries. Of the 71 unfed females dissected from resting collections 68 were virgins. The number of unfed females and males resting inside houses were positively associated (Figure 5) but there was no association in the number of either of these categories with gravid and blood fed insects in resting collections.

**Figure 4**

Number of pairs of *Anopheles funestus* observed relative to the start of swarming, itself co-incident with sunset.

Overall both male and female wing size distributions were approximately normal and were not significantly different (Figure 6). The wing length of males from resting collection, leaving houses, collected from swarms and in copula was similar (Table 1) (Figure 7a). The mean wing size of males differed according to collection site (one-way ANOVA for 2 d.f., $p = 0.037$). Among the females, those collected in light-traps (i.e. host seeking) were smaller than the other categories (Figure 7b). The wing size of females collected in copula were similar to that of newly emerged insects collected resting or exiting from houses.

During the period of study temperatures dropped from an average of 29°C to 26°C. Female wing sizes increased over this period but male size did not (Figure 8).

The mean wing beat frequency of 16 female *An. funestus* collected resting was 516 s.d. 54 Hz. This was similar to the 497 s.d. 16 Hz of the 8 local *An. gambiae* s.l. females. In both species male wingbeat frequencies were higher than the females (706 s.d. 139 for *An. funestus*, $n = 6$ and 781 for the *An. gambiae* s.l., $n = 2$).

Discussion

Swarms of *An. funestus* were so common in Furvela that it is strange that they have not been observed elsewhere. They differed from those recorded by Harper [10] in that they all occurred in very open spaces several metres off the ground rather than the threshold of houses a few centimetres off the floor. *Anopheles funestus* is now known to belong to a group of species, some of which are non-vectors [15] and it is not certain which member of the group the author had observed. The mosquitoes in Furvela were all morphologically *An. funestus*. They also behaved like *An. funestus* in that they were highly anthropophilic and endophilic.

Swarms of male *An. funestus* had an overall appearance similar to those of *An. gambiae* seen in São Tomé [5]. In both species they occurred at sunset 2–4 m above the ground in relatively open areas. Swarm size, dimensions and the estimated number of males per swarm appeared to be relatively constant between sites and species but the number of females entering swarms showed considerable variation. Swarm sites, each of which has a fixed capacity, may determine the dispersal of males. One might expect

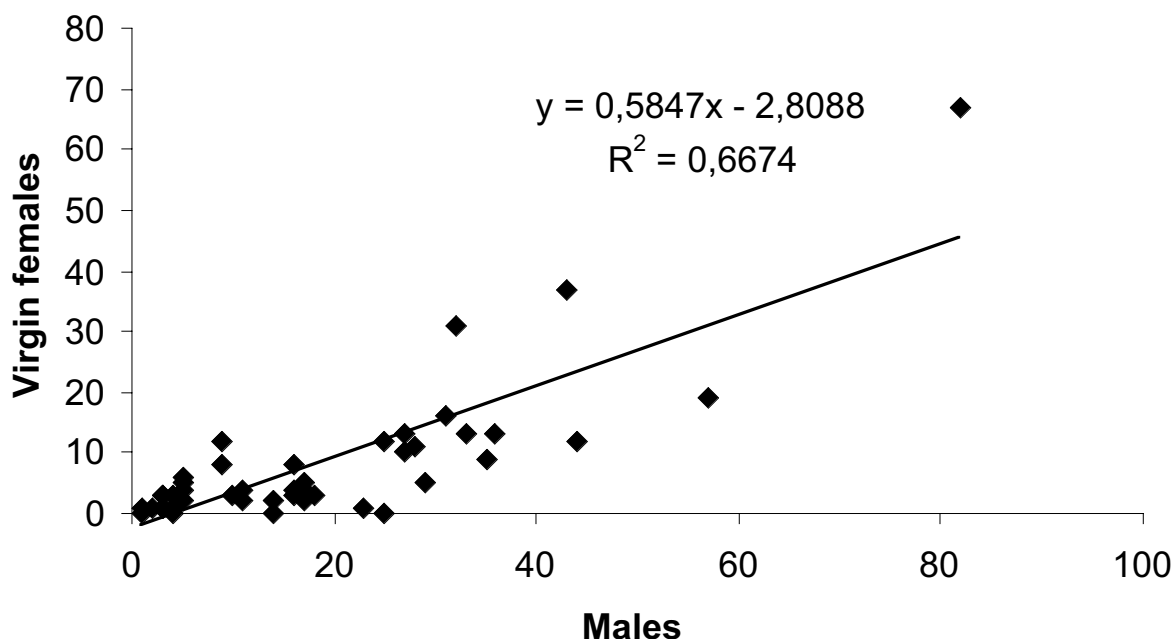


Figure 5

Relationship between the number of males and unfed (virgin) females collected resting inside houses, Furvela village, Mozambique.

relatively more mating to take place in the swarms closest to the female emergence site. We were unable to determine the number of males in a swarm and hence unable to determine if male mating success rates were similar in the different swarms. The fact that male and newly emerged female ratios resting in houses were relatively constant implies that location of swarm has little influence on mating success.

The lack of a relationship between either of these two groups and older females highlights the fact that entry routes, reasons for entry, sense used and time entered differs between the two categories. Males and virgin females use their eyes and enter houses at dawn usually via the darkest orifice (which usually means windows and doors). They come in search of a place to rest. Mated females enter the house at night through the eaves. They are in search of a bloodmeal and use their sense of smell for direction.

With a single exception all swarms were mono-specific for *An. funestus*. Thus, even though the flight tones of female *An. funestus* and the local female *An. gambiae* s.l. were

similar, short range discrimination by male *An. funestus* does not seem to be required since they are only likely to encounter con-specific females. On the one evening when a sample of *An. gambiae* s.l. was obtained two distinct swarms, one of an apparently larger mosquito were seen prior to their being sampled. The swarms occurred circa 50 cm apart, with the larger insects swarming closer to the ground. It is not certain that the species were mixed. Given that they were caught swarming at least once during the study begs the question of why swarms of *An. gambiae* s.l. were not more commonly observed or induced over markers in Furvela as they were in São Tomé [5]. One reason may be that the member of the complex observed in São Tomé was the M form of *An. gambiae* which is not found in Eastern Africa. It would appear to be a different species from the other members of the complex and thus may behave differently. It is perhaps worth mentioning that the artificial markers followed by *An. gambiae* from São Tomé were dark on light [5] whereas those followed by *An. melas* in The Gambia were light on dark [12].

As with *An. gambiae* [3] male size appeared to have little effect on male behaviour. In particular all size groups were

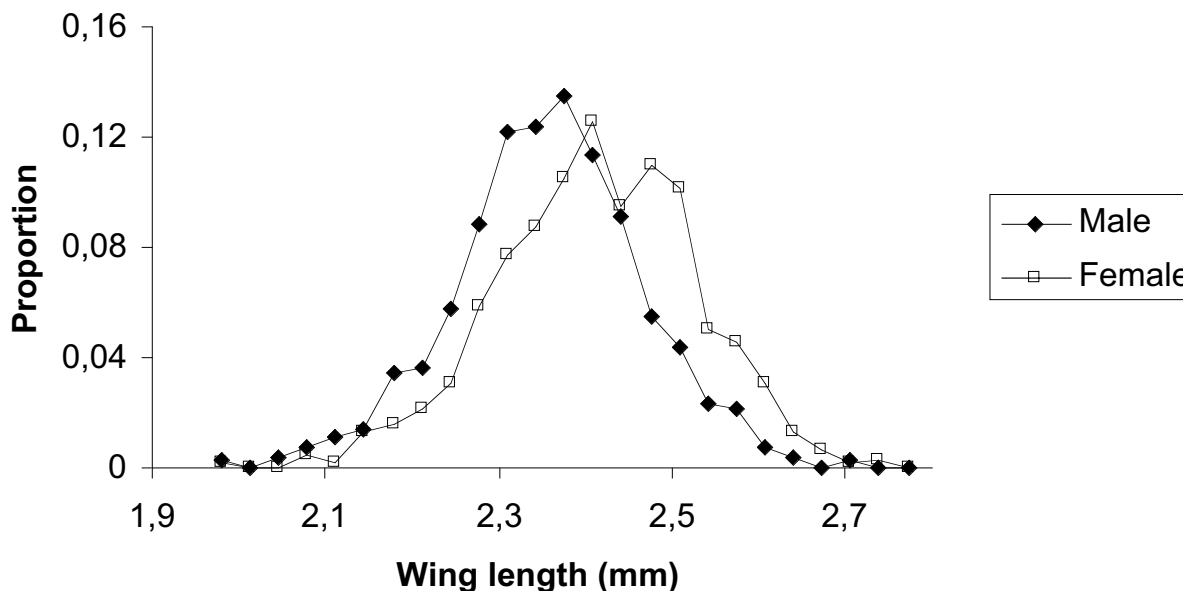


Figure 6
Wing length distribution of male and female *Anopheles funestus* from Furvela village, March-May 2002.

caught in copula, the caveat being that the numbers involved were small. The sampled houses were situated at varying distances from the river valley, the putative breeding site. Differences in size of males according to collection site were small but significant. This may be due to the relatively large sample size or because size influenced male dispersal

Recently it has been shown that in small cages smaller female *An. gambiae* are less likely to mate than larger ones. The authors attribute this to selection by males [16]. One of the difficulties with this interpretation is that a male can inseminate four or more females whilst females are largely monogamous. Given the equal sex ratio at emergence an average male will only meet a single female in his lifetime. If so it would seem unlikely that males would be too selective since there is probably another set of genes around that is not and should a further female come along then she can still be fertilized. Indeed the size distribution of females caught in copula was not significantly different from those emerging.

As temperatures drop the duration of larval and pupal stages increase and the size of emerging insects increases [17]. The duration of the larval stage in *An. funestus* may

be extended by several days as temperatures drop [11]. Male mosquitoes develop faster and emerge earlier than females. The effect of the drop in temperature on mean female size but not male size may be because the adult males collected had not been exposed as larvae, or particularly as pupae [18] to such low temperatures as the females. Further analysis will determine whether mean male size remains the same throughout the year.

Conclusions

Mosquitoes of the *An. gambiae* complex swarm over areas of contrast [5,12]. *An. funestus* avoided such markers (both light and dark) and dispersed if they were placed underneath naturally occurring swarms. The response to potential horizontal markers is likely to be sufficient to prevent mixed swarms from occurring and this is likely to be the factor responsible for isolation between *An. funestus* and the *An. gambiae* complex.

Authors' contributions

Charlwood conducted the work in the field and helped prepare the manuscript

Thompson provided logistical support to the fieldwork

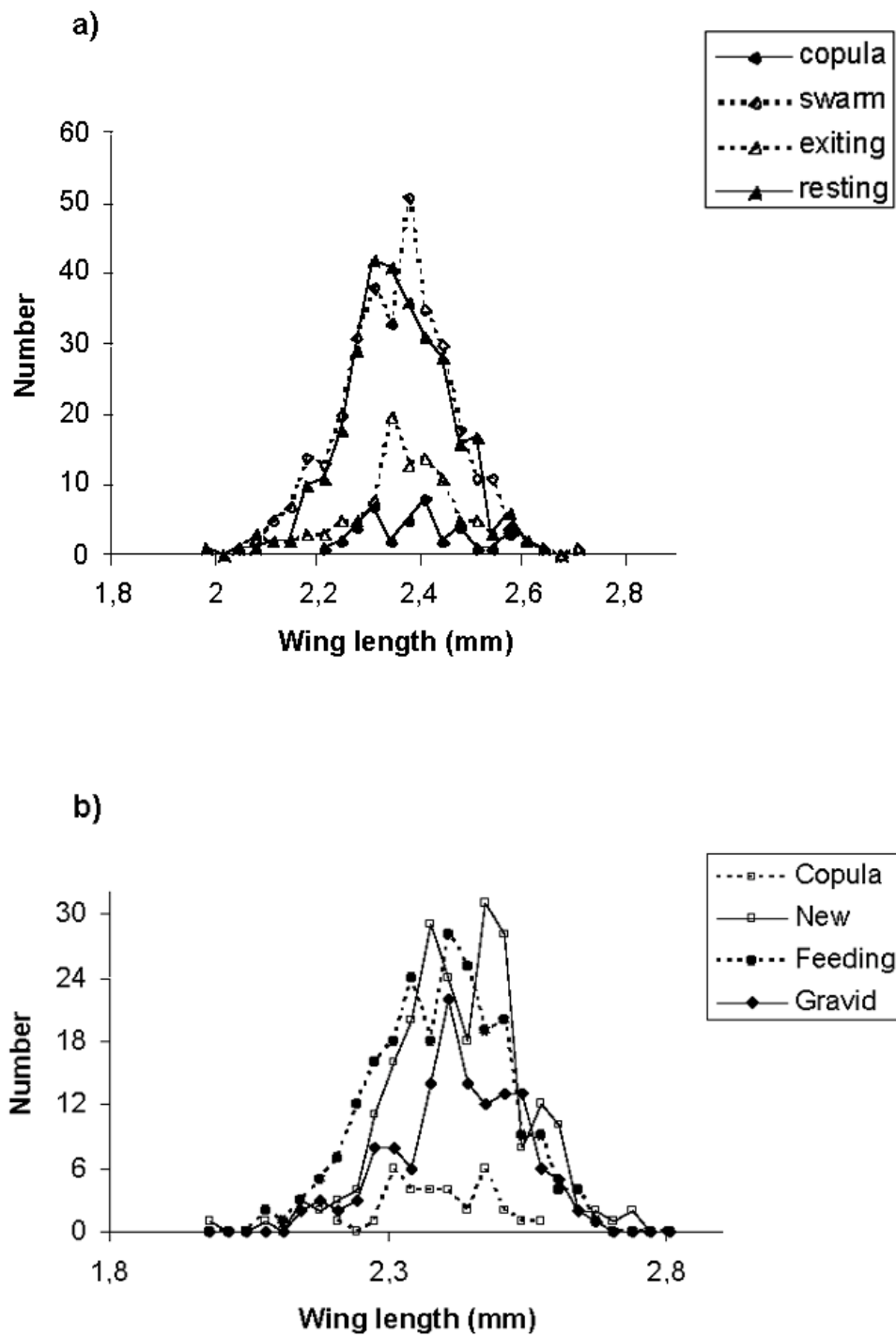


Figure 7
 a) Wing length distribution of male *Anopheles funestus* according to collection method. b) Wing length distribution of female *Anopheles funestus* according to collection method.

Table 1: Winglength of male *Anopheles funestus* collected at different phases of the activity cycle, Furvela village, Mozambique.

Collection Type	Number examined	Mean wing length	s.d.
Resting	298	2.14	0.09
Exiting	112	2.15	0.10
Swarming	328	2.14	0.10
In copula	40	2.17	0.09

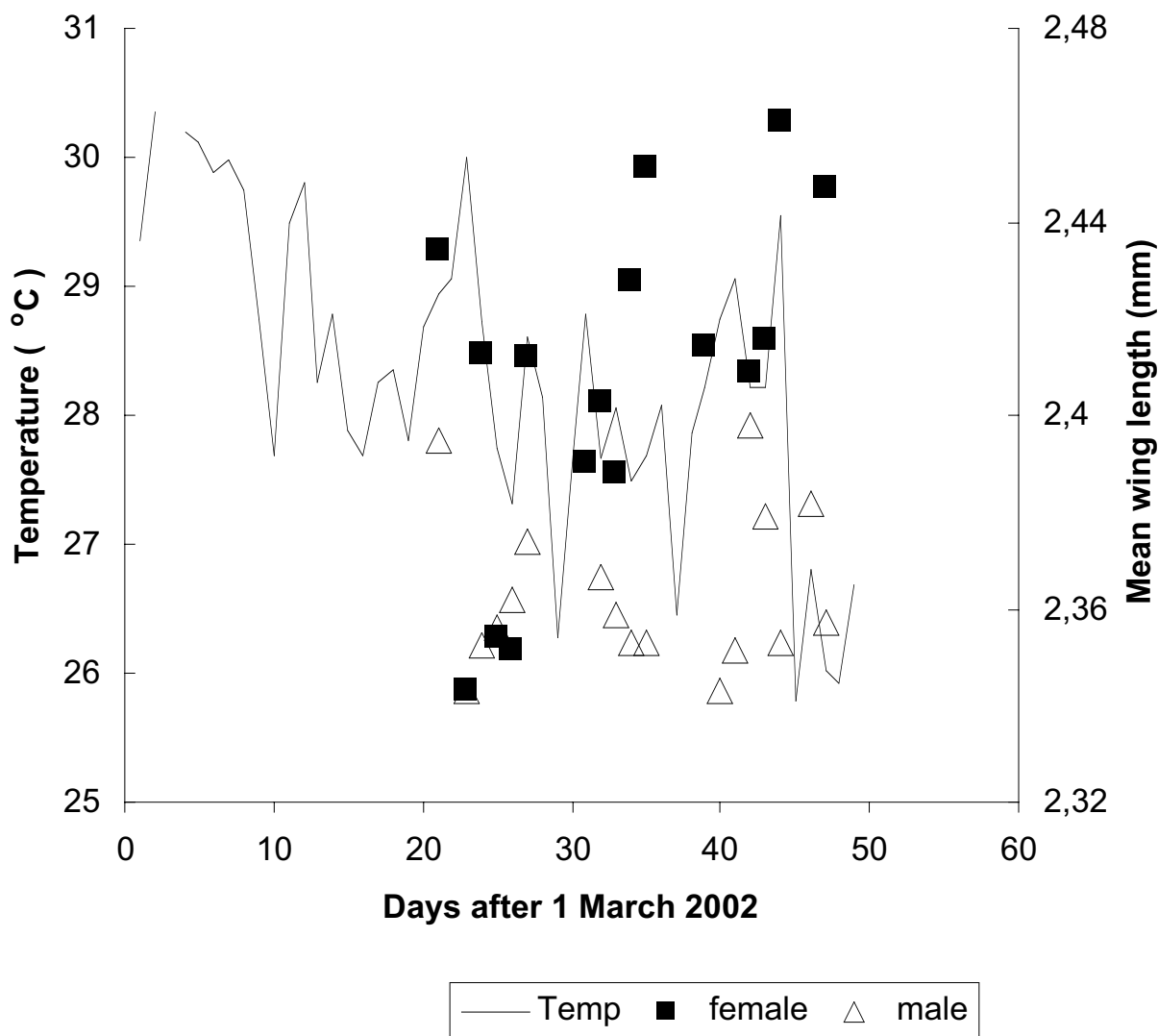


Figure 8
 Mean male and female wing length of *Anopheles funestus* and ambient temperature from Furvela village, Mozambique, by date of collection.

Madsen helped prepare the manuscript, was responsible for figures and provided statistical support

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