# Observations on the swarming and mating behaviour of *Anopheles culicifacies* Giles in nature*

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*The mating and swarming behaviour of* A. culicifacies *Giles was investigated during December 1975 at a cattle shed near the village of Sattoki, Lahore District, Punjab, Pakistan. On average, swarming commenced 20.9 min before sunset (light intensity 1414.4 lx) and ended 21.0 min after sunset (5.4 lx) with pairing restricted to the period from 6.1 min before (467.2 lx) to 15.8 min after sunset (26.9 lx). The swarms were principally composed of males, with females entering only for mating. On average, copulation lasted 27.2 s and was completed in flight. Most females (71.8%) collected while mating had taken a partial blood meal either the previous evening or on the same evening as mating. All females in the swarms were nulliparous and 82.6% had ovaries developed to at least Christophers' stage IIa.*

Adequate knowledge of the swarming and mating habits of mosquitos is of importance in colonization attempts and may have direct relevance in genetic control experiments in assuring the adequate mating competitiveness of the laboratory-reared, released males. The mating and swarming behaviour of *A. culicifacies* Giles has been described only for an outdoor insectary population (*1*). Since genetic control experiments with *A. culicifacies* are planned, we decided to study this behaviour in nature to ensure that the insectary observations were applicable to natural populations, especially since colonization procedures typically alter reproductive behaviour.

## METHODS AND MATERIALS

All observations were made close to the Ghulam Mohammad cattle shed near the village of Sattoki, Lahore District, Punjab, Pakistan, which has been described previously (*2*) and is shown diagrammatically in Fig. 1. During the time of observation, buffaloes and cattle were tethered in front of the feeding troughs (Fig. 1). The elephant grass between the seepage canal and the cattle shed was about 4 m tall and extended above the roof. Fires were often lighted in the compound but the smoke did not appear to interfere with *A. culicifacies* swarming.

Swarms were observed from start to finish against the lighted western sky and were collected, in part, by several sweeps of a net to determine their composition. Pairing times (the time the pair was first observed until the partners separated) were recorded by means of a stopwatch and the direction of the mating flight was noted. Other mating pairs were collected individually with a net, placed in separate tubes, and returned to the laboratory where the trophic condition was scored using the WHO criteria (*3*) and the ovarian condition was graded using Christophers' stages (*4*). Parity was determined by

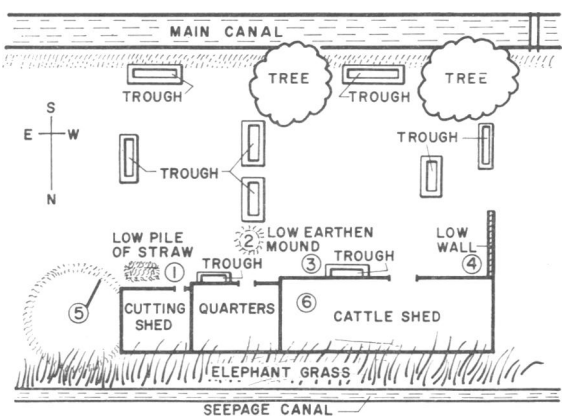


Fig. 1. A diagram of the Ghulam Mohammad cattle shed and its environs with the locations of *A. culicifacies* swarm formations numbered 1 to 6.

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Table 1. Time in relation to sunset (– before and + after sunset) and light intensity at which swarming and pairing began and ended; based on 9 observations

| | Time (min) | | Light intensity (lx) | |
|---|---|---|---|---|
| | mean±SE[a] | range | mean±SE[a] | range |
| Swarming starts | −20.9±1.70 | −11 to −26 | 1414.4±79.4 | 1076.4 to 1614.6 |
| Pairing starts | −6.1±1.49 | 0 to −12 | 467.2±72.8 | 86.1 to 699.7 |
| Sunset | 17 h 03.8 | 17 h 01 to 17 h 08 | | |
| Pairing ends | +15.8±0.92 | +11 to +21 | 26.9± 5.3 | 7.5 to 54.8 |
| Swarming ends | +21.0±0.94 | +15 to +24 | 5.4± 1.4 | 2.2 to 12.9 |

[a] SE = standard error of mean.

the degree of coiling of the ovarian tracheoles (*5*) and insemination was determined by spermatheca dissections.

Temperature, relative humidity, and light intensity were recorded at 5-min intervals between 16 h 00 and 18 h 00 on four evenings using a Taylor hygrometer [a] and a Kahlsico photometer [b] accurate to 0.1 footcandles (≈1.1 lx). Photometer readings were all made at ground level with the photosensitive cell lying parallel with the ground.

## RESULTS AND DISCUSSION

### *Swarm description*

Swarming activity commenced on average 20.9 min before sunset (Table 1) with one or two males flying slowly in circles above a selected portion of the courtyard. Swarms usually formed above low projections such as a mound of dirt or pile of straw (swarms 1 to 5, Fig. 1), but on occasion they also formed at the corner of the cattle shed (swarm 6, Fig. 1). Swarms were consistently present at these locations, but a typical "marker" was not recognized. Swarms never formed above domestic animals or humans, and they formed only in the immediate vicinity of the cattle shed. Usually the swarms were fairly low, 1 to 4 m high, and spherical in shape. The swarms sometimes moved vertically or horizontally, but always returned to the area of formation. The individuals within swarms consistently "faced" a certain direction, usually north-west but occasionally north or west; this is contrary to the findings of Russell & Rao (*1*) whose insectary swarms always faced east.

Swarming at Sattoki began at a much higher light intensity (1414.4 lx, Table 1) than that observed by Russell & Rao, which was "about 2.0 footcandles" (≈21.5 lx). At Sattoki, *A. culicifacies* females bite cattle earlier in the evening during December than they do in August (*2*), and perhaps swarming activity also shifts to earlier in the crepuscular period because of the colder winter temperatures. In the evening, light intensity and temperature dropped rapidly while relative humidity increased markedly

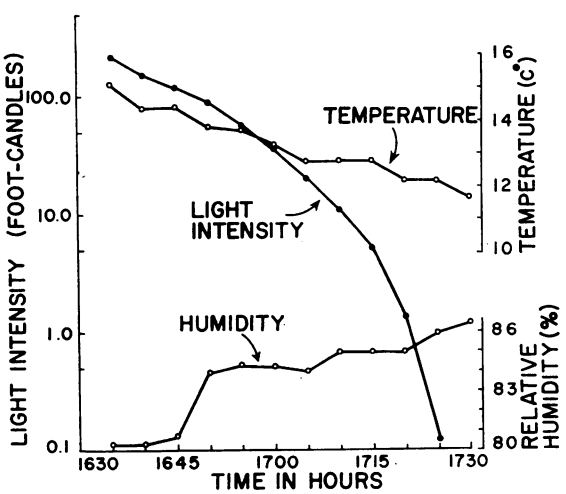


Fig. 2. Change in mean light intensity, temperature, and relative humidity from 16 h 00 to 18 h 00 observed on 8, 10, 12, and 17 December 1975. One footcandle ≈10.7639 lx.

[a] Taylor Instrument Corp., Ashville, NC, USA.
[b] Kahl Scientific Instrument Corp., El Cajohn, CA, USA.

(Fig. 2). Perhaps this rapid temperature and/or humidity change altered the normal response to light intensity. Swarming continued until late twilight (Table 1), ending 21.0 min after sunset with a mean duration of 41.0 min (range = 36–47 min), which was considerably longer than the 2 to 20 min observed by Russell & Rao (*1*).

*Swarm composition*

The composition of samples from 25 *A. culicifacies* swarms collected in six different locations (Fig. 1) around the cattle shed was 544 males and 14 females (ratio = 38.9 : 1), indicating that these swarms were composed essentially of males with females entering the swarms to mate. Apparently attracted to similar micro-ecological conditions or perhaps " markers ", males of *A. stephensi* Liston were collected within five *A. culicifacies* swarms and males of *Culex pipiens fatigans* Weidemann from one, while females of *A. annularis* van der Wulp, *A. nigerrimus* Giles, and *A. pulcherrimus* Theobald were collected once each from *A. culicifacies* swarms. However, females of these species were not inseminated.

*Mating*

Pairing began on average 6.1 min before sunset and continued until about 15.8 min after sunset, the average duration being 21.7 min (range: 15–31 min) (Table 1). After the swarm had completely formed, pairs could be detected flying, in copulation, away from the main body of the swarm. Occasionally, when a female apparently approached the swarm, a group of the males would dart in her direction. Presumably slight changes in the tone of male wing beats stimulated similar behaviour, since many of these " darts " did not result in successful pair formation. Mating was " tip-to-tip ", with the copulating pair leaving the swarm and slowly drifting towards the ground in a " wobbling-type " flight. It was not discerned whether the male or the female led the direction of the flight. The average copulation time for 76 pairs was 27.2 s (standard error of mean = 0.92 s). Copulation was normally completed before the pair reached the ground. Copulation time was considerably longer than the 15 s reported by Russell & Rao (*1*); however, they also observed that the pair usually separated when contacting the screen wall of the insectary or the enclosed vegetation. The direction in which 52 mating pairs flew after leaving the swarm was found to be random with 26.9% flying north, 23.1% south, 19.2% east, and 30.8% west ($\chi^2 = 1.538$, $P > 0.05$). Two pairs were observed to remain within the swarm and fly upwards.

Of 46 mating pairs collected by net and dissected, 28.3% had not fed, 19.6% had recently fed, and 52.2% had fed the previous evening. In 82.6% the ovaries were developed to at least stage IIa and all females were nulliparous as indicated by the tight coiling of their ovarian tracheoles (*5*). Most blood meals were weak or partial feeds and in no instance were the ovaries developed beyond stage II. A single freshly-fed, replete female was observed to remain in copulation for 45 s, falling to the ground before the pair could successfully separate. Another replete female collected by net remained in copulation even after transfer to the collection tube and this pair seemed to experience considerable difficulty in separating. Apparently a replete midgut may be a mechanical hindrance to mating. Blood feeding by unmated *A. culicifacies* females was reported by Russell & Rao (*1*) and was suggested previously by Reisen et al. (*6*) who found unfertilized, freshly-fed females resting in cattle sheds and virgin females feeding on buffaloes. This initial blood meal was apparently used instead of a sugar meal to mature the ovaries to the resting stage II (*5*). Reisen et al. (*6*) also found evidence of multiple blood feeding in *A. culicifacies* with 2 blood meals required, at times, to complete ovariole development. Thus, a given female may take as many as three blood meals from emergence to initial oviposition. *A. culicifacies* is the primary vector of rural malaria throughout much of the Indo–Pakistan subcontinent (*7*), and thus this increased incidence of man–vector contact has considerable epidemiological significance by increasing vector efficiency.

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## RÉSUMÉ

### OBSERVATIONS SUR LE COMPORTEMENT D'*ANOPHELES CULICIFACIES* GILES EN CE QUI CONCERNE LE VOL NUPTIAL ET L'ACCOUPLEMENT DANS LA NATURE

Au cours de décembre 1975, on a étudié le comportement de *A. culicifacies* Giles en ce qui concerne l'accouplement et le vol nuptial dans un abri pour des bovins à proximité du village de Sattoki, district de Lahore, Punjab, Pakistan en vue de vérifier si les comportements décrits par Russell & Rao (1942) pour une population d'insectarium pouvaient être directement extrapolés à des populations sauvages.

Le vol nuptial commençait environ 20,9 min (éclairement 1414,4 lx) avant le coucher du soleil et se terminait 21,0 min après (5,4 lx); il durait 41,0 min. L'accouplement était limité à la période allant de 6,1 min avant le coucher du soleil (467,2 lx) à 15,8 min après (26,9 lx), et durant 21,7 min. En général, la population sauvage de Sattoki semblait effectuer le vol nuptial et s'accoupler plus tôt au cours de la période crépusculaire et pendant plus longtemps que la colonie d'insectarium de Russell & Rao. La copulation durait 27,2 s et s'accomplissait en vol. La plupart des femelles (71,8%) recueillies pendant l'accouplement avaient pris un repas de sang partiel, soit le soir précédent, soit le soir même de l'accouplement. Bien qu'il ne paraisse pas obligatoire, ce repas de sang a été considéré comme pris à la place du repas de sucre normal et comme nécessaire pour fournir l'énergie indispensable au développement de l'ovariole jusqu'au stade II de Christophers. On a estimé que l'ingestion de ce repas de sang supplémentaire accroissait l'efficacité de *A. culicifacies* en tant que vecteur du paludisme, du fait de l'accroissement du nombre des contacts homme-vecteur. Toutes les femelles s'accouplant étaient nullipares et chez 82,6% les ovaires avaient atteint un développement correspondant au moins au stade IIa.


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