

SEASONAL TRENDS OF *ANOPHELES CULICIFACIES*
POPULATION AND ITS SIBLING SPECIES STATUS AT
GOMADIYAGALA- A VILLAGE IN THE
NORTH - WESTERN PROVINCE OF SRI LANKA.

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Abstract

Anopheles culicifacies s.s and *An. subpictus* were the most predominant species found in this study area and accounted for 31.2% and 41.4% respectively of the total mosquitoes collected from the cattle - baited hut collections during the 3-yr period. The relative adult densities of *An. culicifacies* s.s recorded during cooler and rainy conditions were significantly higher than those recorded during hot periods ($p < 0.013$) indicating a tendency to increase the vector density during the cool, rainy seasons. Screening of 364 mosquitoes collected from four sampling methods namely cattle - baited hut collection (CBHC), human baited night collections (HBNC), larval collections (LC) and hand collections (HC) using Rp217 and Rp234 DNA probes showed the absence of sibling species A.

1. Introduction

Anopheles culicifacies Giles, *sensu lato* is the most important vector of malaria in Sri Lanka (Briercliffe, 1935, Wickramasinghe & Samarasinghe, 1991). The taxon *An. culicifacies* exists as four sibling species designated as species A and B (Green & Miles, 1980), species C (Subbarao, et. al., 1983), and species D (Subbarao, et al., 1988). Studies in India have shown that the pattern of distribution and the prevalence of the four sibling species vary in different geographical localities (Subbarao, et. al., 1988). In most areas two or more species have been found to be sympatric with a predominance of one species, while in some areas, only species B has been found. Recently, another two new populations of species B have been identified in Rameshwaram island (Subbarao, et. al., 1993). On the basis of limited cytogenetical studies of *An. culicifacies* populations in Sri Lanka, only species B has been identified so far in the island. (Green and Miles, 1980 ; Abhayawardana, et. al., 1996). Differences in vectorial capacity, seasonal variation, their response to insecticides and biting behaviour have been found among the sibling species of this complex. Therefore, the presence of two or more sibling species in a locality would mask the real transmission potential of malaria and thus, would complicate malaria vector control programmes.

Studies on *An. gambiae* complex (Coluzzi, 1992) and *An. dirus* complex (Baimai, 1988) have shown that some behavioural differences of sibling species have a genetic basis. These genetically different populations of a sibling species would be expected to be at different evolutionary stages of divergence mainly due to adaptation to slightly different micro-environments.

The present study analyses of *An. culicifacies* population in Gomadiyagala in relation to the density during wet and dry seasons and the sibling species status.

2. Materials and Methods

Study Area

Field investigations were carried out in Gomadiyagala, a village in the North Western Province from June 1989 to June 1992. It is situated at the boundary of the Intermediate Dry Zone and about 150 km away from Colombo (Figure 1). The village is surrounded in the East and the West by two mountain ranges. Teak and Eucalyptus plantations, that fall within the Pallekelle Forest Reserve form the Northern and Southern boundaries respectively. The habitat can be considered as a "deforested area" with some larger trees limited to the hilly areas.

The village is inhabited by an approximate population of 200 people. They dwell in more or less semi-permanent structures made of mud walls and thatched roofs. The inhabitants are mostly engaged in 'chena' (slash and burn) cultivation.

A tributary of the Hakwatuna Oya runs through the village joined by two small streams. These water sources form the main permanent breeding habitat for anophelines.

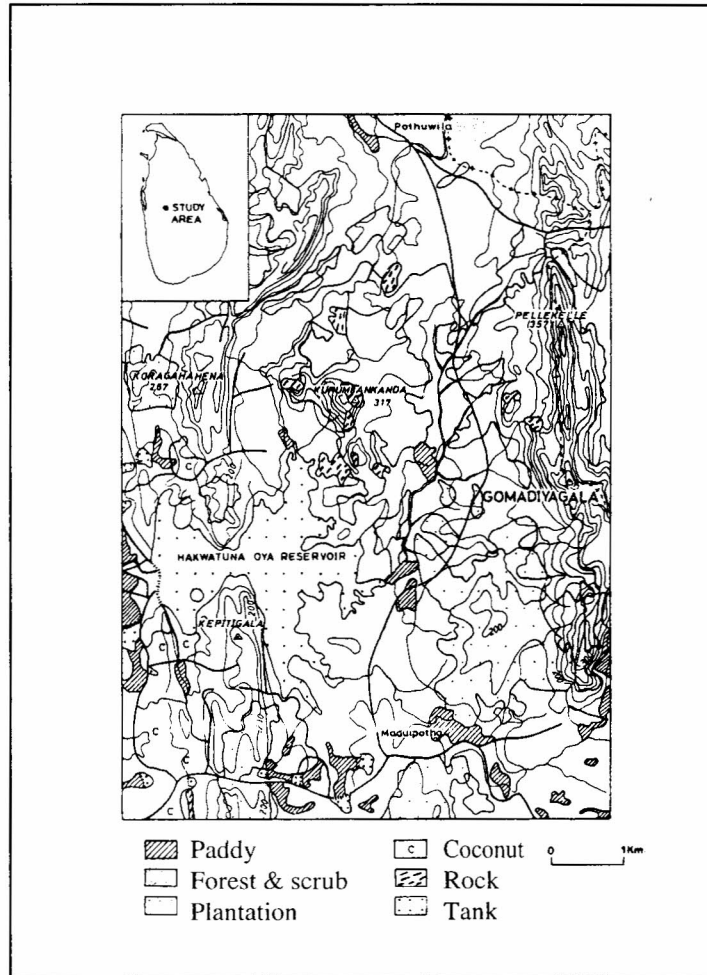


Figure 1: Map of study area. The inset shows its position in Sri Lanka.

Meteorological data

A rain gauge was set up in the locality to measure the rainfall. Temperature and relative humidity were recorded using a maxi-mini thermometer and a hair hydrometer respectively.

Sampling of *An. culicifacies* s.s. adults from cattle baited hut collection (CBHC)

Adults were collected from two cadjan huts (210 x 210 x 180cm) constructed about 500m apart. Mosquitoes resting inside the huts were collected by two collectors using an aspirator and a torch light over a 15 minute period before dawn.

A standard anopheline key (Carter, 1949) was used for the identification of *An. culicifacies*. During the latter part of this investigation the sibling species status of *An. culicifacies* collected from Gomadiyagala was analysed using the DNA probe method (n=364) and the cytogenetic method (n=25). The average number of anopheline mosquitoes per hut for each month (monthly geometric mean GM_{MI-12}) was calculated as given below.

$$GM_{MI-12} = (Y_1 + Y_2 + \dots + Y_n)^{1/n}$$

Y = Number of endophilic mosquitoes per hut.

M = Months of the study period.

In addition, the overall geometric mean (GM_o) of the 3-Yr period was also calculated as given below.

$$GM_o = (Y_1 + Y_2 + \dots + Y_N)^{1/n}$$

Deviation of the monthly mean from the overall mean was plotted to depict the pattern of seasonal abundance.

Identification of sibling species

Single mosquito DNA extractions were carried out as described by Collins et al, (1987) and a 1/200 th dilution of each DNA sample in TE buffer (10mM Tris, 1mM EDTA, pH 8.0) was dot blotted on to nitro-cellulose filters and the dot blots were prehybridized for 16h at 37 °C in prehybridization buffer (1M NaCl, 50% formamide, 1% SDS, 10% dextran sulphate, 50 mM Tris-HCl pH 7.6, 100 µg/ml heparin) and hybridized for 16h at 37 °C with ³²p Oligolabelled (Feinberg and Vogelstein, 1983) Rp234 (Rep 340) and Rp217 (Rep 217) DNA probes (specific activity 10⁹cpm/µg DNA). The final wash of the filters was carried out at 30°C in 0.1 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate) and the air-dried filters were autoradiographed (Kodak XAR-5 film with an intensifying screen) for 16h at -70°C.

3. Results

Meteorological information

The data collected on temperature, rainfall relative humidity during the study period are given in Figure 2. The annual rainfall recorded in the area was 1664 mm and 1249 mm in 1990 and 1991 respectively. Rainfall is associated mainly with the North-East monsoon which prevails from October to January each year. The average rainfall per month during the North-East monsoon and post North-East monsoon (February to May) was 235.6 mm and 59.9 mm respectively. A monthly average of 50.1 mm of rainfall was recorded during the South-West monsoon. The mean

maximum and minimum temperatures were $31.0 \pm 0.9^{\circ}\text{C}$, $21.5 \pm 0.9^{\circ}\text{C}$ respectively in the North-East monsoon period (wet period), $34.0 \pm 1.3^{\circ}\text{C}$, $22.7 \pm 1.7^{\circ}\text{C}$ respectively in the post North-East monsoon period and $31.5 \pm 1.4^{\circ}\text{C}$, $23.6 \pm 0.5^{\circ}\text{C}$ respectively in the South-West monsoon period (Dry period). The relative humidity ranged from 70.5% ($\pm 14.7\%$) during the North-East monsoon period and 72.8% ($\pm 5.2\%$) in the South-West monsoon period.

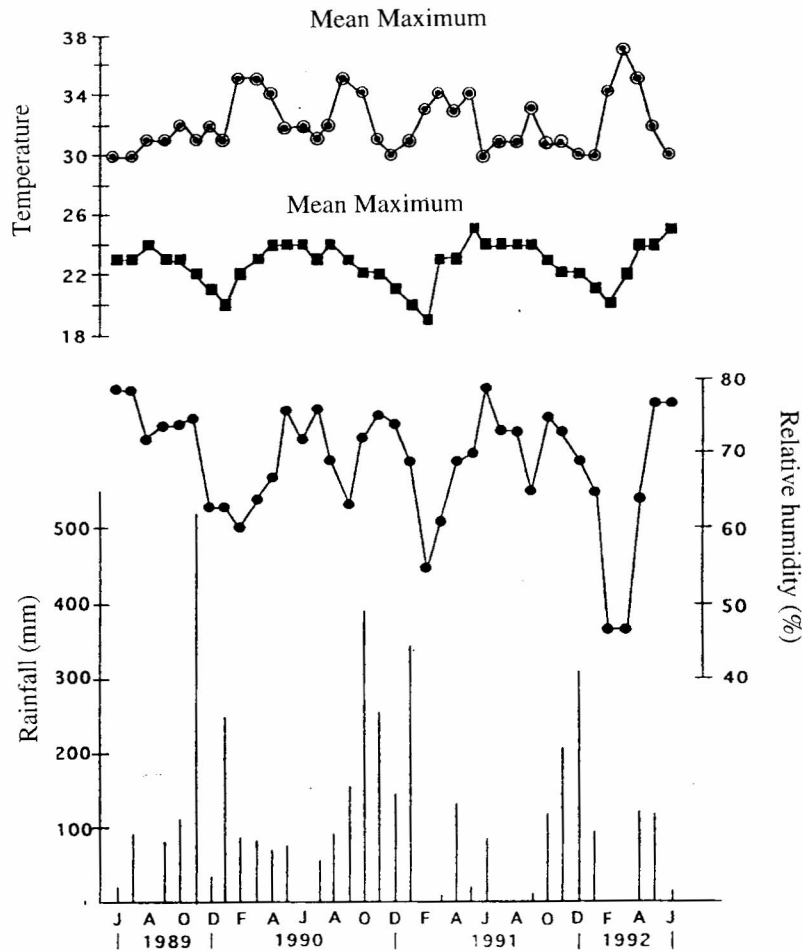


Figure 2: Changes in the monthly mean of maximum and minimum temperature ($^{\circ}\text{C}$), the percentage relative humidity read at 0800 h and total rainfall (mm) in Gomadiyagala from June 1989 to June 1992.

***An. culicifacies* s.s relative abundance**

An. culicifacies s.s and *An. subpictus* were the most predominant species and reported 31.2% and 41.4% respectively of the total mosquitoes obtained from cattle-baited hut collections during the 3-yr period. Table 1 shows the number (percentage) of different anopheline species obtained from cattle-baited hut collections from June 1989 to June 1992. To depict these seasonal abundance pattern, the deviation of the monthly mean mosquito density of *An. culicifacies* s.s from the Overall Mean

Table 1: Total anopheline mosquitoes collected at Gomadiyagala from June 1989 to June 1992.

Species	Catches from cattle-baited huts
<i>An. subpictus</i>	11,129 (41.4%)
<i>An. culicifacies</i>	8,304 (31.2%)
<i>An. hyrcanus</i>	2,230 (8.4%)
<i>An. vagus</i>	2,150 (8.1%)
<i>An. barbirostris</i>	1,575 (5.9%)
<i>An. varuna</i>	960 (3.6%)
<i>An. pallidus</i>	178 (0.6%)
<i>An. annularis</i>	107 (0.4%)
<i>An. tessellatus</i>	25 (0.09%)
Total	26,658

(Geometric Mean) was plotted. (Figure 3). It was observed that the adult density of *An. culicifacies* was relatively low during August to September (dry period) whilst a considerably high density was recorded during January - April. This coincides with the onset of the North-East monsoon.

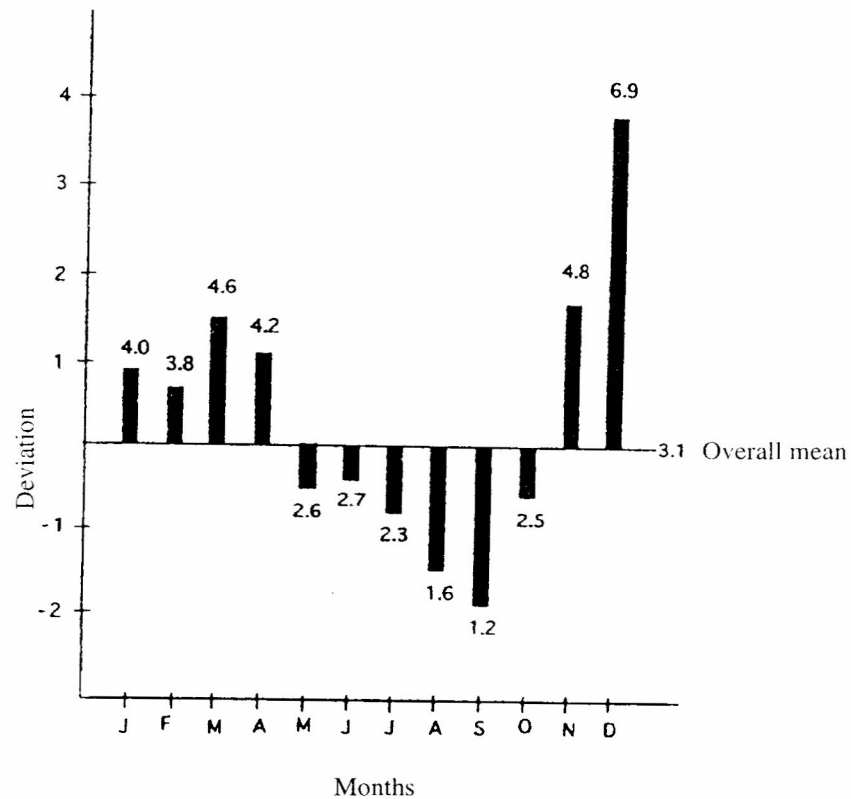


Figure 3: The pattern of seasonal abundance of female *An. culicifacies* s. collected from cattle - baited huts at Gomadiyagala from June 1989 to June 1992. The number of mosquitoes collected was standardized for 15 minutes per hut of collection effort, transformed to $\ln(Y+1)$, and averaged over months (Geometric mean). Overall geometric mean was also calculated as above using the data collected during the 3-yr period. The deviation of the monthly geometric mean from the overall mean is plotted.

Sibling species status

All 364 *An. culicifacies* mosquito DNA samples from mosquitoes collected by four sampling methods gave a positive hybridization signal with Rp234 and Rp217 when diluted 200 fold and assayed by the Dot-blot hybridization (Figure 4). The mosquito collection period (March 1991 - June 1992) covered the hot/dry and cool/rainy periods of the year would enable to collect one or more sibling species of *An. culicifacies* if they were present. These results indicate that the non-existence of *An. culicifacies* A in the *An. culicifacies* population at Gomadiyagala.

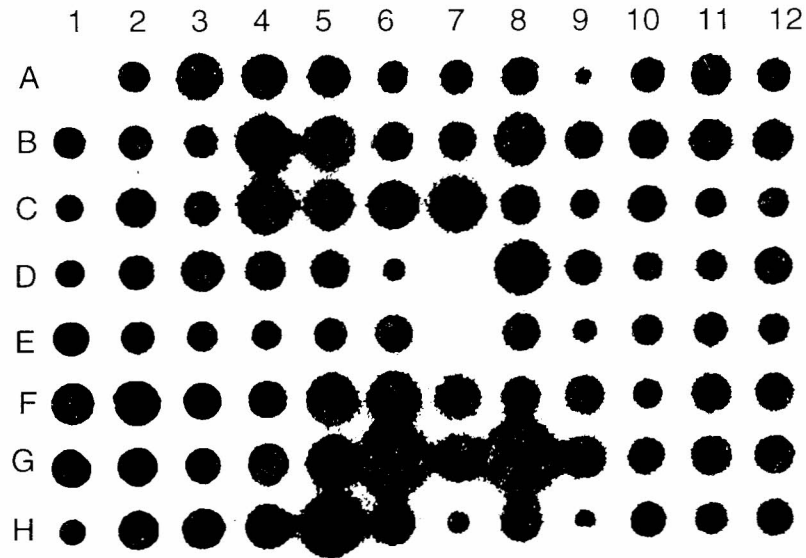


Figure 4: Dot-blot hybridization of single mosquito DNA extracts (diluted by 200 fold) of wild caught mosquitoes with Rp 234 DNA probe (Rp217 gives an identical hybridization pattern).

Control DNA samples:

An. culicifacies sibling species B (India) H1 and D6.

An. culicifacies sibling species A (India) A1 and D7.

An. tessellatus (Sri Lanka) E7.

All wild caught mosquitoes gave a positive hybridization signal.

4. Discussion

The density of *An. culicifacies* s.s increased during the North-East monsoon period and fluctuated throughout the post North-East monsoon period (January - April). A relatively low density was recorded during the South-West monsoon (average rainfall was 50.1 mm) which was observed to be not very conducive for the breeding of *An. culicifacies*. Therefore, the distribution of *An. culicifacies* was unimodal with peaking around the North-East monsoon periods during November-April. The relative adult densities recorded during cooler conditions were observed to be significantly higher than those recorded during hot periods ($p < 0.013$) indicating a tendency to increase the vector density during the cool, rainy seasons.

Investigations in India have shown that sibling species A of *An. culicifacies* is dominant in the hot season while species B predominates during the rainy season. It has also been revealed that sibling species A is the major vector of malaria in most

parts of India while species B acts as a poor vector (Suguna, et al., 1983). However, there appears to be some behavioural differences between *An. culicifacies* species B found in India and Sri Lanka. Although initially only sibling species B was recorded from Rameshwaram island, more recently two types of sibling species B (RAC-Rameshwaram acrocentric, RSM- Rameshwaram sub metacentric) based on the mitotic karyotype of Y chromosome have been noted in the island (Subbarao, et al., 1993). These observations are indicative of slight evolutionary divergence between *An. culicifacies* sibling species B found in other regions of India and in Rameshwaram island, which may have resulted due to isolation and adaptation to different micro-environments. Since species B found both in Sri Lanka and in Rameshwaram island acts as a major vector of malaria, it is also a possible that a similar variation exists in *An. culicifacies* found in Sri Lanka.

We have previously shown (De Silva, et. al., 1993; Gunasekera, et. al., 1995) that Rp36, Rp217 and Rp234 repetitive sequences could be used as DNA probes to detect *An. culicifacies* from other mosquito species as well as to distinguish species A from B and C using a 200 fold dilution of a single mosquito DNA extract in a Dot-blot hybridization assay.

Screening of 364 mosquitoes collected from Gornadiyagala - a malaria endemic area in Sri Lanka has consistently shown the non-existence of *An. culicifacies* sibling species A. Twenty five semi-gravid mosquitoes collected from Gornadiyagala were screened for sibling species status using cytogenetic method. All these mosquitoes belonged to sibling species B (Abhayawardana, et. al., 1996).

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