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# GONOTROPHIC CYCLE OF *Aedes (Stegomyia) Aegypti* (Linnaeus) (DIPTERA: CULICIDAE)

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**ABSTRACT**

Gonotrophic cycle of *Aedes (Stegomyia) aegypti* (Dengue vector) was studied under controlled conditions in the laboratory. The experiments were performed at constant temperatures (± 2°C) of 30° C and 35° C and natural humidity on the females and males obtained from the first generation of laboratory culture. For this study, two - three days old virgin female was placed singly in the rearing cage with a male for mating purpose. Twenty-five such pairs of *Aedes aegypti* were used in the experiment, at each above-mentioned conditions. Blood meal was offered for the maturation of eggs, and multiple mating by a male was allowed. The Facility for the oviposition was provided for each female mosquito. The length and number of gonotrophic cycles ($GC_S$), parous number and fecundity (eggs deposition) during the lifetime of each female mosquito were recorded daily. After death, each female was dissected out to observe eggs remained in the ovaries. The statistical data were analysed with the help of SPSS (20.0 version) Software to follow the length of GC and fecundity according to the temperature. Variations in the duration of gonotrophic cycles were observed. There was a significant difference in the mean duration of GCs, since $P \leq 0.05$. The mean number of eggs produced by each female was also variable according to the temperature and a significant difference in the fecundity of triparous females, since the $P \leq 0.05$. But there was not a significant difference ($P \geq 0.05$) in the fecundity of uniparous and biparous females of *A. Aegypti*. Maximum three $GC_S$ were completed by the female *Aedes (Stegomyia) aegypti* (triparous) and some were observed as nulliparous (no gonotrophic cycle completed by these females). The present investigations indicate that Gonotrophic cycle is temperature dependent. The variations in the temperature play a very important role and they may impact on the vitellogenesis up to a certain limit. This study will provide the additional tool to implement the control strategies for dengue vector at an appropriate time in the Agra region.

Keywords: *Aedes (Stegomyia) aegypti*; gonotrophic cycle; temperature; fecundity; female mosquitoes.

## INTRODUCTION

*Aedes (Stegomyia) aegypti* is a primary vector for transmission of dengue virus and chikunguinya virus in the Agra region. The transmission of dengue viruses (DENV) and chikunguinya viruses (CHIKV) to human by the vector is achieved through the blood feeding [7]. In the course of its adult life the female mosquito mates with a male after their emergence and start looking for a blood meal to obtain the necessary amino acids for egg maturation which lasts a few days [8]. Then the female oviposits in a

suitable breeding site and looks for another blood meal, but this pattern is not so strict for *Aedes (Stegomyia) aegypti* mosquitoes that can take multiple blood meals at any time of egg development [9,10].

Gonotrophic Cycle (GC) of a mosquito is a reproductive cycle which starts at the blood meal and ends with egg laying. It continues till the end of female life and temperature dependent [1,2]. The minimum time estimated for development of mature eggs after blood feeding was 3.5 days. The length of the gonotrophic cycle was estimated as every 4 days and 3 days at 26.7 ± 1.22℃ and 29.8 ± 1.47℃ respectively [3]. To become infective the female of *Aedes (Stegomyia) aegypti* should ingest virus during the bloodsucking and that female will not be able to transmit the virus during another blood meal until the extrinsic incubation period is completed. Consequently, the aged female may carry more infectious risk. During their blood meals, *Aedes (Stegomyia) aegypti* females can ingest the virus that shall disseminate into the mosquitoes body by passing first into the midgut, crossing the intestinal barriers, amplifying into the haemocoel and eventually reaching the ovaries and the salivary glands. The infective cycle starting with the ingestion of the virus and ending when the virus reaches the salivary gland is called the extrinsic incubation period and it is also temperature dependent [4,5]. Both Gonotrophic Cycle and Extrinsic Incubation Period (GC and EIP) are thus taking place in the body of mosquito simultaneously but with different time durations and different temperature-dependent responses [2,6].

The study aimed:

1. to describe the maximum gonotrophic cycles completed by each female of *Aedes (Stegomyia) aegypti*.
2. to provide an additional tool to implement the control strategies for the dengue vector at an appropriate time in the Agra region.

## MATERIALS AND METHODS

The study was performed at Agra region, Uttar Pradesh, India. First generation (F1) of male and female *Aedes (Stegomyia) aegypti* was used in this study, obtained from a laboratory colony, established from the field-collected mosquitoes. The mosquitoes were transferred in the rearing cage with the help of mouth Aspirator. The rearing cages were made of plastic containers and maintained at approximately 30°± 2℃, 35°± 2℃ temperature and natural humidity (variable), which are close to the natural conditions in Agra region during the rainy season. The laboratory temperatures were monitored with a digital thermometer. Albino rat blood meals and sucrose solution were offered to female and male mosquitoes respectively for colony maintenance. Powdered fish food was added for the larval maintenance.

Experiments on the gonotrophic cycle of *Aedes (Stegomyia) aegypti* were performed by using twenty-five pairs of virgin females and males (two to three days old) at each above-mentioned condition. Each female was singly placed in the rearing cage with a male mosquito for mating purpose. After mating, the male was taken out from every rearing cage, and each female was fed upon Albino rat for a blood meal. For the oviposition, each female was allowed to lay eggs in the rearing cage holding 500 ml beaker which was half filled with water. Two wooden pieces were placed on the surface of the water in each beaker as the site for the oviposition. After three days, beakers were taken out and examined for the presence of eggs. After removing eggs, beakers were again placed in the rearing

cages for the observation of the further presence of eggs and just after completing GC, each female was allowed to mate with a new male, blood meal and oviposition facilities were provided in the same manner. The number of eggs and GC completed by each female was recorded. After completing their lifespan every female was dissected out to see the ovarian development and remaining eggs in the ovaries.

Statistical analysis was performed with the help of SPSS 20.0 Software [11]. The raw data (Tables 1-2) were transformed to analyse the mean duration of gonotrophic cycles and deposition of eggs etc. Analysis of variance performed by the use of Levene's test. If the significance value of Levene's test $p \geq 0.05$ then we may be assumed that the variance of the two samples is equal. The t-test was used to estimate the significance of the relationship between temperature, duration of $GC_S$ and number of eggs laid by per female of *Aedes aegypti*.

Null and alternative hypotheses: Null hypotheses for two cases may be formed as follows - $H0_1$: There is no significant difference between the length of gonotrophic cycles at 30℃ and 35℃ temperature. $H0_2$: There is no significant difference between the fecundity of the female mosquitoes at 30℃ and 35℃ temperature.

Alternative hypotheses for two cases may be formed as follows – $H1_1$: There is a significant difference between the length of gonotrophic cycles. $H1_2$: There is significant difference in the fecundity of female mosquitoes.

## RESULTS AND DISCUSSION

The results from the above study show that the duration of the first GC (GC1) was considered as the number of days between the first blood meal and the first egg laying. After the first egg laying, a second blood meal was provided. The duration of the second GC (GC2) was the number of days between the second blood meal and the second batch of eggs deposition. The same method was followed for the third GC (GC3). The duration of GC varied with the temperatures (Table 1). For GC1, the mean duration of 6.35 days was observed at 30℃ temperature and mean 4.59 days at 35℃ temperature. Since $P \leq 0.05$, therefore mean duration of GC1 is not the same at both temperatures. For GC2, the mean duration of 5.58 days at 30℃ temperature and mean duration was 3.25 days at 35℃ temperature. For this GC2 the $P \leq 0.05$ and indicates that there is a significant difference in the length of GC2 of *Aedes aegypti*. For the third GC, mean duration of 4.91days at 30℃ temperature and of 3.27days at 35℃ temperature shows the duration of GC3 was significantly different according to the temperature ($P \leq 0.05$) in the given Table 1. The longer mean duration for each GC i.e. GC1, GC2, GC3 was observed on 30℃ temperature and shorter on 35℃.

The number of GCs completed by each female *Aedes* (*Stegomyia*) *aegypti* varied from one to three gonotrophic cycles at 30℃, 35℃ ± 2℃ temperature and natural humidity. All the females were not able to complete more than one gonotrophic cycle. The percentage of females having 3GC (triparous) was 40% (10 out of 25), 2GC (biparous) was 32% (8 out of 25) and only one GC (uniparous) was 16% (4 out of 25) and 12% was nulliparous (3 out of 25) at 35°C ± 2°C temperature. At 30°C ± 2°C temperature, the percentage of females having 3GC (triparous) was 12% (3 out of 25), 2GC (biparous) was 44% (11 out of 25) and only one GC (uniparous) was 32% (8 out of 25) and 12% was nulliparous (3 out of

25). The high percentage of triparous females was observed at 35℃ ± 2℃ temperature Fig. 4 (A and B).

The number of eggs deposited by each female *Aedes (Stegomyia) aegypti* varied greatly for triparous females but not for uniparous and biparous females at 30℃, 35℃ ± 2℃ temperature (Table 2). The mean number of eggs laid by 4 females (out of 25) was 24.25 and by 8 females (out of 25) was 23.75 at 35℃ and 30° ± 2℃ temperature respectively. These females were uniparous and there was not a significant difference in the fecundity of female *Aedes aegypti* because of the P ≥ 0.05. For biparous females, the mean number of eggs deposited by 11 females (out of 25) was 59.18 and by 8 (out of 25) females was 64.87 at 30℃, 35℃ temperature which again shows that there was not a significant difference in the egg laying, since P ≥ 0.05. For triparous females, the mean number of eggs deposited by 3 (out of 25) females was 62.00 (at 30℃) and by 10 (out of 25) females was 86.00 (at 35°) (Table 2). This is also showing the significant difference (p ≤ 0.05) in the egg deposition during their lifetime. In this experiment, 3 females (out of 25) were nulliparous even not a single egg deposited by them on both temperatures. The mean number of eggs deposited by triparous females was high at 35℃ temperature while it was low at 30℃ temperature.

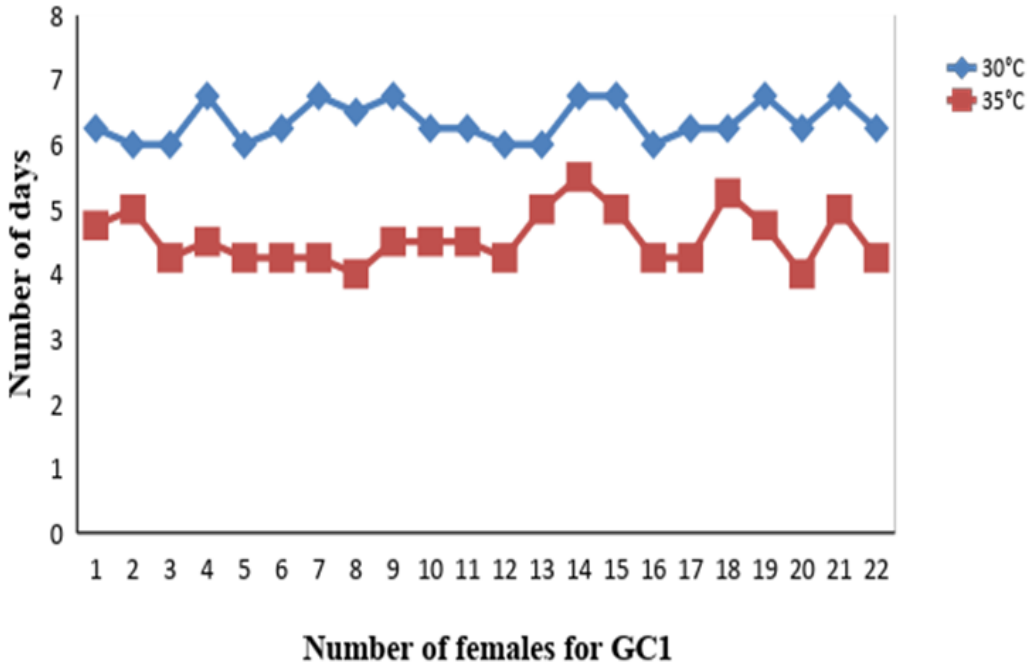


**Fig. 1. Length of GC1 completed by females of *A. aegypti* at 30℃ and 35℃ ± 2℃ temperature**

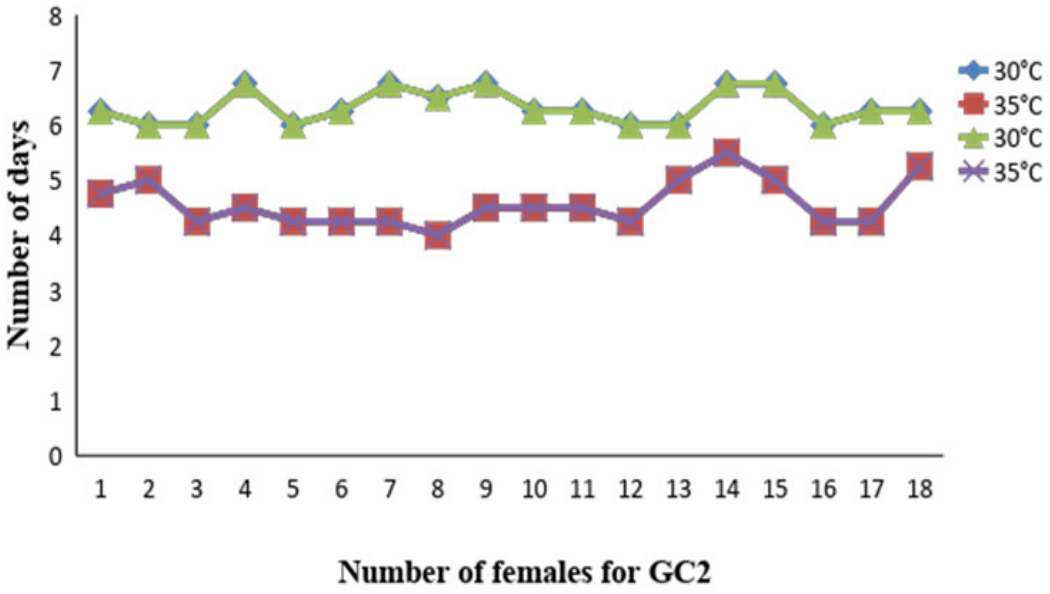


**Fig. 2. Length of GC2 completed by females of *A. aegypti* at 30℃ and 35℃ ± 2℃ temperature**

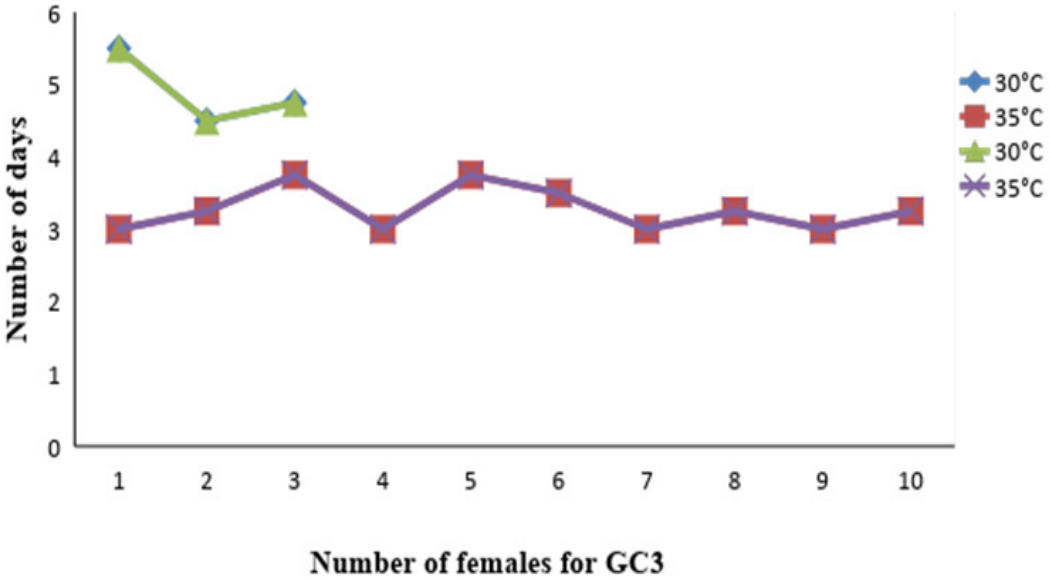


**Fig. 3. Length of GC3 completed by females of *A. aegypti* at 30℃ and 35℃ ± 2℃ temperature**

**Table 1. T- test results (SPSS Software) for the durations of GCs at 30°C and 35°C ± 2°C temperature**

| GCs | T°C | N | D | S.D | SED | T-VALUE | DF | MD | P value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| GC1 | 30 | 22 | 6.3523 | .30530 | .11059 | 15.927 | 42 | 1.76136 | .000 | Reject $H0_1$ |
| | 35 | 22 | 4.5909 | .41936 | | | | | | |
| GC2 | 30 | 14 | 5.5821 | .21716 | .09173 | 25.423 | 30 | 2.33214 | .000 | Reject $H0_1$ |
| | 35 | 18 | 3.2500 | .28440 | | | | | | |
| GC3 | 30 | 3 | 4.9167 | .52042 | .23043 | 7.124 | 11 | 1.64167 | .000 | Reject $H0_1$ |
| | 35 | 10 | 3.2750 | .29930 | | | | | | |

*Note: T= Temperature, N= Number of females observed for respective GCs, D = Mean no. Of days, S.D. = St. deviation, SED = St. error difference, T-value = value of t- statistics, DF = Degree of freedom, MD = Mean difference, P value = significance value Reject $H0_1$ = mean duration of GCs is not same (P ≤ 0.05) at 30°C and 35°C ± 2°C temperature*

**Table 2. T- test results (SPSS Software) for the mean number of eggs deposited by per female during its life time at 30°C and 35°C ± 2°C temperature**

| Parity class | T°C | N | E | S.D | SED | T-VALUE | DF | MD | P value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| Uniparrous | 30 | 8 | 23.7500 | 6.18177 | 4.9984 | -.100 | 10 | -.50000 | .922 | Accept $H0_2$ |
| | 35 | 4 | 24.2500 | 11.52895 | | | | | | |
| Biparous | 30 | 11 | 59.1818 | 6.16146 | 4.11383 | -1.384 | 17 | -5.69318 | .184 | Accept $H0_2$ |
| | 35 | 8 | 64.8750 | 11.66726 | | | | | | |
| Triparous | 30 | 3 | 62.0000 | 6.0000 | 8.70167 | -2.793 | 11 | -24.3000 | .018 | Reject $H0_2$ |
| | 35 | 10 | 86.3000 | 14.33760 | | | | | | |

*Note: T= Temperature, N= Number of females observed for respective parity, E = Mean no. Of eggs, S.D.= St. deviation, SED = St. error difference, T-value = value of t- statistics, DF = Degree of freedom, MD = mean difference, Reject $H0_2$ = mean number of eggs laid by per female during its life time is not same (P 0. ≤ 05) at 30°C and 35°C ± 2°C temperature*

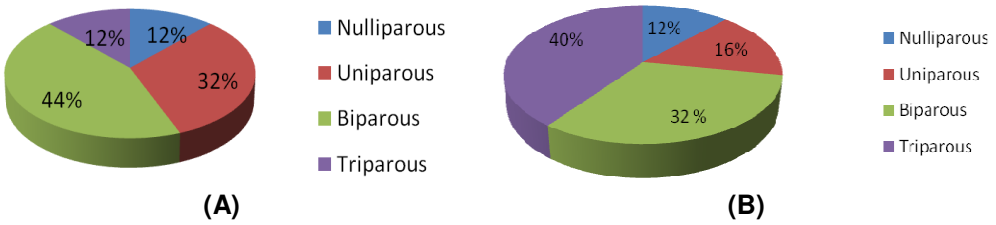


**Fig. 4. Percentage of female *Aedes (Stegomyia) aegypti* mosquitoes according to parity class (A: at 30℃ and B: at 35℃ ± 2℃ temperature)**

## CONCLUSION

The fecundity of *Aedes (Stegomyia) aegypti* will impact on the population density and from the above results, the best performance regarding to gonotrophic cycles, fecundity and percentage of triparous was observed at 35℃ ± 2℃ by the females. Results obtained may have important implications for the current and future distribution of *Aedes (Stegomyia) aegypti* in Agra region. They may serve as a useful indicator of trends in the population with ambient temperature in nature. Thus it will help to control the population of dengue vector at the appropriate time in Agra region, Uttar Pradesh, India.

The present investigations indicate that Gonotrophic cycle is temperature dependent. The variations in the temperature play a significant role, and they may impact on the vitellogenesis up to a certain limit. Many of the females may lay eggs without, and some reveal the process of nulliparity. The factors responsible for this phenomenon are not known. Further investigations on these two aspects may be highly useful to develop control strategies for this vital vector.

## ETHICAL DISCLAIMER

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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