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**Ekezie Chisom C**Department of Parasitology and  
Entomology, Nnamdi Azikiwe  
University, Awka, Nigeria**Aniefuna Chikodili O**Department of Parasitology and  
Entomology, Nnamdi Azikiwe  
University, Awka, Nigeria**Obiakor Ugochukwu A**Department of Parasitology and  
Entomology, Nnamdi Azikiwe  
University, Awka, Nigeria**Umeanor Benjamin C**Department of Parasitology and  
Entomology, Nnamdi Azikiwe  
University, Awka, Nigeria**Corresponding Author:****Ekezie Chisom C**Department of Parasitology and  
Entomology, Nnamdi Azikiwe  
University, Awka, Nigeria

## Sex-specific development causes patterned emergence in *Culex* mosquito

**Ekezie Chisom C, Aniefuna Chikodili O, Obiakor Ugochukwu A and Umeanor Benjamin C**

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### Abstract

In order to understand why adults of the *Culex* mosquito emerge in a manner that follows a well-defined mathematical pattern depending on the sex of the species, preliminary investigations were carried out to determine if sex-related differences exist in the rate of growth and development in the immature stages. *Culex* egg-rafts sampled from the natural breeding environment were hatched in the laboratory and a total of 583 first instar larvae were allowed to grow in isolation. The time taken for the isolated mosquitoes to molt into the next stage was recorded until the mosquitoes finally became adults. At the final molt, the sex of the mosquitoes was determined and assigned retrospectively to every molting stage. Of the entire isolated mosquito, 464 (220 males and 244 females) were able to reach maturity. We observed that the developmental process occurring in the immature mosquito is sex-specific between the fourth instar larva and pupa. This event gives male *Culex* approximately one-day advantage over females and explains why males always emerge first and dominate the first few days during the emergence period. The process also alters the pre-existing symmetry in the molting time distribution of the female mosquito. The outcome is a pattern that could be described as a logarithmic function of the emergence time in male *Culex*, and a power function of the emergence time in female *Culex*. We recommend an investigation of the other species of mosquito as well as other holometabolous insects to see if this observation can be reproduced.

**Keywords:** Sex-specific, development, pattern, emergence, *Culex*

### 1. Introduction

In a recent study that observed *Culex* mosquitoes under laboratory conditions, it was found that adults of the species emerge from their pupa in a manner that is not random but follows a well-defined mathematical pattern. The pattern of emergence is dependent on the sex of the emerging mosquito. Male mosquitoes emerge in a pattern that could be described as a logarithmic function of the emergence time, while female mosquitoes emerge in a pattern that could best be described as a power function of the emergence time both at twenty-four hours intervals<sup>[1]</sup>.

The natural clusters in which *Culex* mosquitoes lay their eggs provided the researchers with an opportunity to breed and observe them in the laboratory. Their objectives were to determine whether offspring from a single parent emerge, from pupa to adult, in a non-random manner and to describe any observed trend. Non-linear regression analysis was used to fit a mathematical model on the data collected. The trend in male *Culex* was found to approximate a logarithmic function with 95% accuracy<sup>[1]</sup>. It is a natural logarithm of the emergence time at twenty-four hours intervals. Natural logarithms are logarithmic functions to the base 'e' where 'e' is approximately 2.718281. Theoretically, the graph of a natural logarithm grows to positive infinity as X increases and slowly goes to negative infinity as X approaches zero<sup>[2]</sup>. In female *Culex*, the data fits the power function model with 92% accuracy<sup>[1]</sup>. A power function is a function with a single term that is the product of an actual number, a coefficient and a variable rose to a fixed power which is also an actual number. With some power functions, as is the case with the emergence in female *Culex*, as X approaches positive infinity, the output Y becomes a huge positive number<sup>[3]</sup>. Following the description of the observed trend, the researchers hypothesized that a sex-based difference might exist in the rate of growth and development of the immature stages. As proposed by the researchers, the implications of a patterned emergence are: that it reduces the chances of successful mating among offspring of the same parent. However, the chance of successful mating with offspring produced by different parents is increased.

Thus, the pattern promotes genetic diversity within the species [1].

The present study attempts to investigate the cause of this observed pattern. It is based on the hypothesis that sex-related differences exist in the growth rate and development of immature *Culex* mosquito. It predicts that if such differences are real, we are expected to see a difference in molting time and molting time distribution in any or every stage in the lifecycle of the mosquito.

## 2. Materials and Methods

### 2.1 Source of Mosquito Egg

Eggs of *Culex* mosquito collected from their natural breeding environment were used. A sampling of freshly laid eggs was done eight times during the investigation period, which spanned from August 2020 to November 2021.

### 2.2 Sampling of Mosquito Larvae

All egg-rafts collected were hatched in universal sample containers. From every egg hatched at any particular period of time, not less than forty (40) first instar larvae were sampled. On the first period of egg hatching, forty-four (44) first instar larvae were isolated from the population. The second up until the eight periods had 55, 71, 75, 78, 115, 89, 56 larvae isolated respectively. The samples were pooled together to give a total of 583 mosquitoes.

### 2.3 Isolation and Growing of Mosquito

All 583 first instar larva used in the study were isolated from the rest within six hours after hatching. The larvae were picked, under a bright light, with a pasture pipette. Each larva was transferred to a 15cm test-tube and allowed to grow independent of others. This was done by carefully dropping a single larva close to the edge of the test-tube and washing it down with 3ml of distilled water. All isolated larvae were given a number tag and fed with a pinch of powdered yeast. Cotton wool was used to plug the top of every test-tube.

### 2.4 Observation of Growth in the Mosquito

The observation was made at six hours intervals to measure the time taken for each larva to molt from one stage to another until they finally emerge into adult mosquitoes. The cast-off exoskeleton was used as an indicator for the end of one growth stage and the beginning of another growth stage. All mosquitoes observed to have molted at every period of 6hr, had their identification numbers, molting time and molted stage recorded. It should be noted that observation of the first two molting stages was done under bright light, using a magnifying glass to enable one to see the shed exoskeleton.

### 2.5 Assigning Sex to the Immature Stages of the Mosquito

At the end of the growth period for a particular mosquito, i.e. when the mosquito has emerged to adult, the sex of the mosquito was determined and recorded for that identification number. The sex was also assigned retrospectively to every molting stage for the same identification number. For

example, if the mosquito in test-tube number 2 and 15 emerge as female and male respectively, then 'F' and 'M' is retrospectively assigned to 2 and 15 respectively from the pupa, fourth instar, third instar, second and first instar larva.

## 2.6 Data Analysis

The mean molting time for both males and female and the 95% confidence interval were computed for the different molting stages. Also the median (Q2), upper quartile (Q3) and lower quartile (Q1) for the two sexes was computed for the different molting stages. Box and whiskers as well as line graphs were plotted using Microsoft Excel version (2016). The degree of skewness of the different distributions from the third molt was calculated using Bowley's coefficient of skewness given as:

$$Sk = \frac{(Q3 - Q2) - (Q2 - Q1)}{(Q3 - Q2) + (Q2 - Q1)}$$

Where the value of Sk ranges from -1(perfect negatively skewed) to +1 (perfect positively skewed). Sk= 0 implies a perfect symmetrical distribution.

Kurtosis for the different distributions from the third molt was calculated using Karl Pearson's Gamma coefficient given as:

$$\gamma_2 = \frac{\mu_4 - 3\sigma^4}{\sigma^4}$$

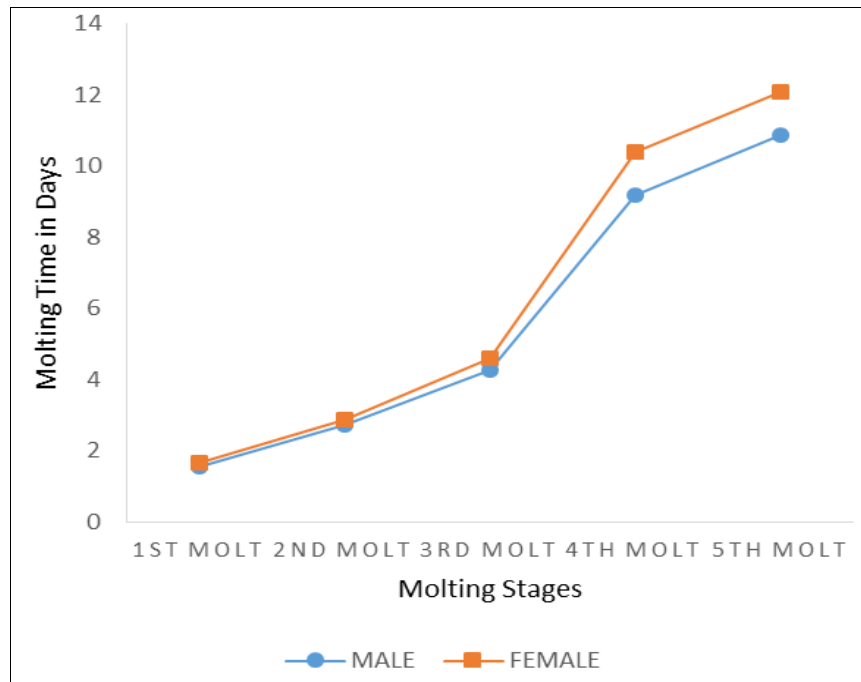
Where  $\mu_4$  is the fourth moment about the mean and  $\sigma$  is the standard deviation. The value of  $\gamma_2$  ranges from  $\gamma_2 < 0$  (Platy-Kurtic) to  $\gamma_2 > 0$  (Lepto-Kurtic).  $\gamma_2 = 0$  implies a normal or Meso-Kurtic curve.

F-Test for equality of variance was done to determine if the variability in molting time for male and female *Culex* differ significantly in all the molting stages ( $\alpha = 0.01$ ).

## 3. Results

Out of the 583 first instar larvae that was sampled, 464(80%) were able to reach maturity. This comprised of 220 (47%) male mosquitoes and 244(53%) female mosquitoes.

It took the male mosquitoes an average of  $38 \pm 1$  hours to molt for the first time after hatching. After the first molt, it took the same sex another  $28 \pm 2$  hours to molt a second time. Movement from the second to third molt took  $37 \pm 3$  hours, from third molt to pupa took  $118 \pm 9$  hours while from pupa to adult took  $41 \pm 1$  hours. For female mosquitoes, it took an average of  $40 \pm 1$  hours to molt for the first time after hatching. After the first molt, it took another  $29 \pm 2$  hours to molt a second time. Movement from the second to third molt took  $42 \pm 3$  hours, from third molt to pupa took  $139 \pm 11$  hours, while from pupa to adult took  $41 \pm 1$  hours. Notice that the difference in time taken for male and female mosquitoes to move from fourth instar larva to pupa is 21 hours ( $\approx 1$  day). See Figure 1 for the mean molting time of the two sexes of mosquito in days.



\* The slight difference in the 2<sup>nd</sup> and 3<sup>rd</sup> molt was due to chance. This was confirmed using F-Test for equality of variance.

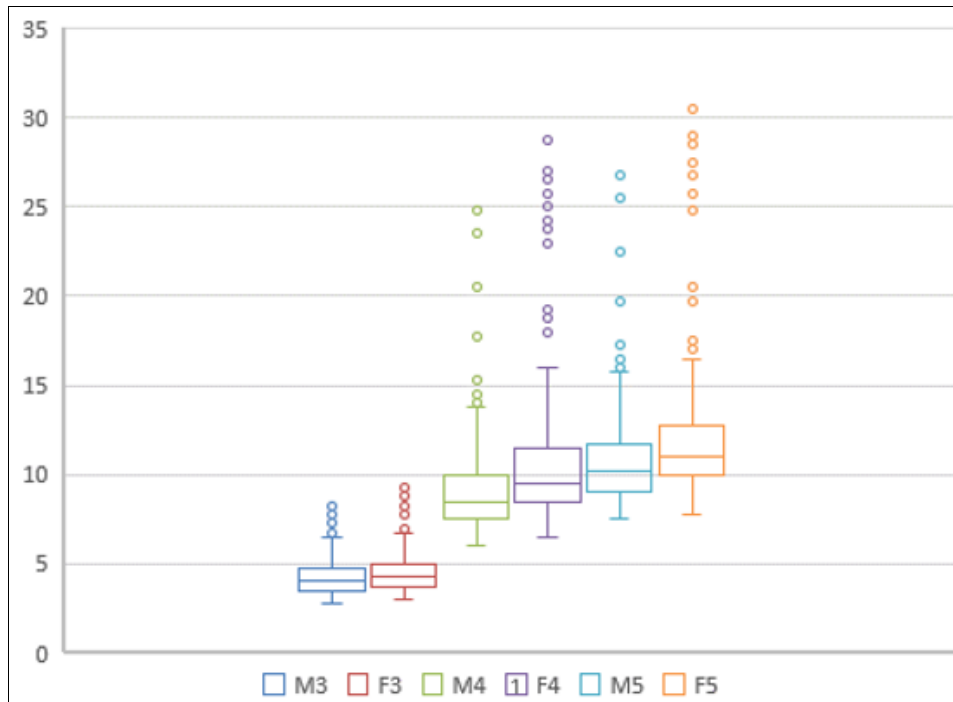
**Fig 1:** Shows the mean molting time of both male and female mosquitoes in days

The result of an F-test for equality of the variance observed in the molting time of male and female *Culex* is give as follows: for the first molt [ $F=1$ ,  $F_{(0.01)}(219,245)= 1.39$ ,  $P>0.01$ ]; for the second molt [ $F=1.29$ ,  $F_{(0.01)}(219,245)= 1.39$ ,  $P> 0.01$ ]; for the third molt [ $F=1.12$ ,  $F_{(0.01)}(219,245)= 1.39$ ,  $P> 0.01$ ] and fourth molt is [ $F=1.92$ ,  $F_{(0.01)}(219,245)= 1.39$ ,  $P< 0.01$ ]. When variations due to fourth molt were removed from fifth molt before testing for equality of variance for the fifth molt, the result is [ $F=1.11$ ,  $F_{(0.01)}(219,245)= 1.39$ ,  $P>0.01$ ]. From this we conclude that the variability in the molting time for both male and female is the same in the first three and fifth molting stages but differ significantly only on the fourth molting stage.

Figure 2 is a side by side box and whisker plot that compares the distribution of male and female molting time from the third molt to the fifth molt. The first pair of boxes represents the distribution of male and female molting time respectively after the third molt. Notice that the boxes are almost identical. The middle line dividing the box into two half (median or Q2) is almost at the centre in the two boxes. Thus, the coefficient of skewness for the two distribution is  $Sk= 0.2$ . The second pair of boxes represents the distribution of male and female molting time respectively after the fourth molt. Notice that the lower length of the fourth box (i.e. the lower quartile or Q1 of the female distribution) begins at a point similar to the middle line of the third box (i.e. the median or Q2 of the male distribution). This observation reflects the difference in molting time observed in figure 1. Another thing worthy of note is while the median of the third box remains unchanged (i.e. the same as in the first two boxes) the fourth box has its

median slightly closer to the lower quartile. Thus, the coefficient of skewness for the distribution of the male molting time after the fourth molt remains  $Sk = 0.2$ , while the coefficient of skewness for the distribution of female molting time after the fourth molt is  $Sk=0.3$ . For the third pair of boxes, which represents the distribution of male and female molting time after the fifth molt, the pattern observed is similar to the second pair of boxes except that the median of the fifth box becomes even more central. Thus, the coefficient of skewness for the male distribution after the fifth molt is  $Sk=0.1$ , while the coefficient of skewness of the female distribution remains  $Sk=0.3$ . The dots seen above every box represents outliers which affects the mean molting time. Bowley's coefficient does not take into account these outliers and this is why it is the best estimator of skewness for this kind of distribution.

Since Bowley's coefficient of skewness excludes outliers, they were removed when computing the Karl Pearson's Gamma coefficient for kurtosis. Note that outliers are defined as any point above or below [ $1.5 \times (Q3-Q1)$ ]. After the third molt, the kurtosis for the distribution of both male and female molting time is  $\gamma_2= 0.3$ . The kurtosis after the fourth molt is  $\gamma_2= 0.1$  for male *Culex* and  $\gamma_2= -0.1$  for female *Culex*. Kurtosis after the fifth molt is  $\gamma_2= 0.5$  for male *Culex* and  $\gamma_2= 0.3$  for female *Culex*. Gamma coefficient can take any value greater than or less than zero, but because our calculated values are closer to zero (although not perfectly) we conclude that all the distributions are meso-kurtic or have a normal kurtosis. Thus, we can say that kurtosis of the molting time distribution was not altered by the growth of the mosquito.



\*\*The upper length of the box represents the upper quartile (Q3); the lower length of the box represents the lower quartile (Q1); the middle line inside the box represents the median (Q2); the dots above the box are outliers; the vertical line above and below the box is the highest and lowest value that is not an outlier; M3 and F3 are male and female after the third molt.

**Fig 2:** Compares the distribution of male and female molting time from the third molt to the fifth molt

#### 4. Discussion

*Culex* mosquito molts five times in its life time. During the first three molts, the larvae increase its size but do not change its form. On the fourth and fifth molt, the larvae change its form to become pupae and pupae become adults. When allowed to grow in isolation, it was observed that both male and female of the species undergo a similar developmental process during the first three molting stages. Between the third molt and pupation, the developmental process becomes sex-specific. This was evident in the large difference observed in the average time taken for both sexes to move from fourth instar larva to pupa; and also in the significant difference that exists in the variability of the molting time observed after the fourth molt in both sexes (refer to the second and third paragraph as well as figure 1 in the result section). The sex-specific process gives male *Culex* approximately one-day advantage over females of the same species and helps explain why the male mosquitoes dominated the first 48 hours of emergence observed in Ekezie *et al.* [1]. Development from pupa to adult took the same average time in both male and female, suggesting that the process is similar in both sexes.

An outcome of this sex-specific event is a 'molting time' distribution which is more symmetrical in male *Culex* and more asymmetrical in females of the same species. Because we cannot directly observe what is being developed between the fourth instar larvae and pupae, we can at least predict its characteristics by studying the distribution of the time taken for the development to complete. It is a fact that all populations of plants and animals show variation in observable characteristics and when these traits are quantitative they do not show clear cut differences between individuals but form a spectrum of phenotypes which results in a continuous variation [4] and [8]. A continuous variation is a symmetrical or what is often called a normal distribution. A normal distribution is one in which majority of the cases fall

in the middle of the scale and the small number of cases is located at both extremes of the scale. The first and third quartiles of a normal distribution are equidistant from the median; the mean, median and mode (if it is uni-modal) fall at the same point; the distribution is meso-kurtic. Any deviation from these properties is termed skewness or asymmetry [6]. We found that both male and female had these properties, of a normal distribution, before pupating. After the fourth molt, the symmetry remained unchanged in male *Culex* but was altered in female *Culex* (see paragraph four and five as well as figure 2 in the result section). The difference in symmetry of the molting time distribution after the fourth molt suggests that whatever feature(s) that has developed at this stage, when identified and measured, will show a continuous variation that is more symmetrically distributed in male *Culex* or more asymmetrically distributed in female *Culex*. Therefore, this difference explains why male emergence is a logarithmic function of the emergence time and female emergence is a power function of the emergence time [1].

To demonstrate further the relationship between symmetry of the distribution of molting time and the emergence model a mosquito will follow, let us examine the equation of emergence of both sexes of the mosquito. When a variable is increased by adding a number to it, the value for the mean, median and mode will go up by the number added, thus the symmetry will remain unchanged. On the contrary when a variable is raised to a power, the symmetry of the distribution changes [7]. From the equation for the emergence of male *Culex* given as  $Y=13.183\ln(x) + 23.164$  [1], we noticed that a constant is added to the natural logarithm of the emergence time ( $x$ ) to increase the number of emerging mosquito ( $Y$ ). This implies that the distribution is most likely to be symmetrical. For female *Culex*,  $Y=2.2925(x)^{1.2758}$  [1], we saw that the emergence time ( $x$ ) was raised to a power to increase the number of emerging mosquitoes ( $Y$ ) and this implies that

the distribution is positively skewed (asymmetrical). Challenges encountered during the study include the loss of follow-up (due to mortality) in about twenty percent of the samples either in the early stages or before the final stage of growth. Also, there was a little difficulty in finding the shed exoskeleton during the first two molts. However, the latter was overcome by shaking the test-tubes gently and observing them under a bright light using a hand lens. We recommend an investigation on the other species of mosquito as well as other holometabolous insects to see if the same observation occurs in them. Also biochemical changes that occur in both male and female *Culex* as they grow from fourth instar larva to pupa needs to be studied. There is also a need to understand the implication(s) of the asymmetry in molting time distribution to the biology of female *Culex* mosquitoes.

## 5. Conclusion

In an attempt to understand why *Culex* mosquito emerge following a well-defined mathematical pattern which is dependent on the sex of the species, this study was conducted to determine if there exist sex-related differences in the rate of growth and development of the immature mosquito. Our result demonstrated that somewhere between the fourth instar larva and pupa, the developmental process occurring in the immature mosquito becomes sex-specific. This event gives male *Culex* approximately one-day advantage over females and also alters the pre-existing symmetry in the development time of the female mosquito. The outcome is a pattern in which males of the species emerge rapidly in the beginning and slows down as time increases, while the number of female emerging is slower at the beginning but rises sharply as time increases.

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