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Patterned emergence in *Culex* mosquitoes: Evidence, hypothesis, and insights on its consequences in a complex breeding environment

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Abstract
Culex mosquitoes usually lay their eggs in form of a raft with up to 100 to 400 eggs. This eggs hatch at the same time to release first instar larva which molts four times, pupate and finally become adults. In the present study, it was observed that emergence of adults follow a well-defined pattern which depends on the sex of the emerging mosquito. The pattern followed by emerging males could be described as a logarithmic function of the emergence time at 24 hours interval. Female emergence could best be described as a power function of emergence time when compared to the exponential function model, both at 24 hours interval. A difference in the rate of growth and development which may exist between the male and female immature stages of the species was proposed as a cause of the pattern. Insights from the data shows that the pattern reduces the frequency of successful mating that may occur between offspring of same parent hatched at same time. In a complex breeding environment, there is a higher chance that successful mating will occur between offspring of different parents hatched at different time, thus it could be said that the pattern promotes genetic diversity within the species.

Keywords: culex mosquito, emergence, logarithmic, power function

1. Introduction

Mosquitoes deposit two basic types of eggs: rapid-hatch and delayed hatch. Rapid-hatch eggs are deposited directly into water, on the surface, or on substrate close to the water and usually hatch within 48 hours. The rapid-hatch eggs are laid individually, in small groups, or in rafts containing up to several hundred eggs [1].

Culex mosquitoes lay their eggs in the form of egg rafts that float in still or stagnant water. The mosquitoes lay their eggs one at a time sticking them together in the shape of a raft which contains from 100 to 400 eggs [2]. A raft of eggs looks like a speck of soot floating on the surface of water and is about ¼ inch long and 1/8 inch wide [3].

Eggs hatch to release the first instar larvae which molts four times. At the 4th instar, the usual larva reaches a length of almost ½ inch and towards the end of this instar ceases feeding. When the fourth instar molts, it becomes a pupa. The metamorphosis of the mosquito into an adult is completed within the pupal case. The adult mosquito splits this case and emerges to the surface of water where it rests until its body dries and hardens [3].

In the present study, *Culex spp* were observed to metamorphose into adult mosquitoes and emerge in a manner that is not random. The aim of this article is to describe the observed trend in emergence; to hypothesize why such trend occurs, and finally to explain the consequences of this pattern in a complex breeding environment.

2. Materials and Methods

2.1 Sampling and Hatching of Eggs

A total of about twenty (20) egg rafts of *Culex spp*, sampled from the wild, were hatched separately in universal containers. All larvae that were observed to have hatched from a single egg raft at any particular time were transferred into a 500ml Erlenmeyer flask after two days, and were given an identification number.

Soft mosquito nets (free from insecticides) were used to cover the mouth of each flask. The larva were fed with yeast and bred to adult.

2.2 Emergence and Counting

The flasks were monitored every day until emergence began to take place in each of them. Counting of the number of emerged mosquitoes in any particular flask was done at 24hrs interval starting from the time the first mosquito was observed to have emerged in that flask. With the help of chloroform, the mosquitoes were knocked down, sexed (separated into male and female) and counted using Right-Way^{TM} hand tally counter. All necessary precautions were observed while using chloroform. This includes ensuring that the immature were not killed by the reagent.

2.3 Data Analyses

Data was presented as the cumulative frequency of both male and female mosquito that was observed to have emerged from each flask at every 24hrs interval. In other to understand the pattern in the data, a scatter-plot was created from the average of all male as well as female emergence data; and non-linear regression analyses (trend analysis) was used to fit a model on the data points using Microsoft Excel version 2016.

3. Result

Observation of emergence at twenty-four hours interval shows that the number of male mosquitoes emerging at any particular time increases rapidly at the beginning of the emergence period and later slows down with increase in time. Relative to the male of the species, the number of female emerging increases slowly at the onset of emergence and becomes rapid with increase in time (see Table 1). Notice, from table 1, that emerging males consistently dominated the first 48hrs of the emergence period. The number of females was higher than males at the end of the emergence period in some cases but never at the beginning.

The scatterplot shown in figure 1 visually compares the trend in male and female emergence. This was gotten from the average of all twenty data points. Least square regression lines (trend lines) were fit between data points from both sexes to observe the trend in emergence. The trend in male could be described as a logarithmic function of the emergence time; with the equation $Y = 13.183\ln(x) + 23.164$ and trend line reliability, R^2, 0.95 or 95% agreement between line and data. Female emergence could be described as a power function or power regression of the emergence time; with equation $Y = 2.2925x^{1.2738}$ and trend line reliability, R^2, 0.92 or 92% agreement between line and data (see fig 1 for all equations and reliability coefficients). It is worthy of note that the value for 'x' in the above equations is the emergence time at 24hrs interval while 'Y' is the number that emerged at a particular time interval.

Figure 2 compares the exponential function model with the power regression model on female data. Analysis shows that the exponential function model is a poor fit for the female data, with equation, $Y = 6.6054e^{0.1602x}$ and trend line reliability, R^2, 0.64 or 64% agreement, when compared with the power regression model which fits 92% with the data. Note that the thickest line in fig 2 represents the exponential trend line.

Table 1: Showing Sex-based Frequency of Emerging *Culex spp* at 24 hours Interval

S/N	sex	24h	48h	72h	96h	120h	144h	168h	192h	216h	240h	264h	288h	312h	336h	360h	384h	>384h
1	M	14	15															
	F	04	09															
2	M	06	07	08	08	09												
	F	02	04	05	05	05												
3	M	27	41	88	89	90												
	F	00	02	45	78	86												
4	M	13	28	33	38	39	42	42	42	45	47							
	F	03	09	17	25	27	35	39	42	51	56							
5	M	17	80	102	104	105	105	105										
	F	00	00	04	48	77	80	82										
6	M	21	27	38	40	47	48	48	54	55	55							
	F	03	09	15	16	27	30	37	38	39	40							
7	M	24	36	60	68	79	83	91	95	99	99	99	99	99	100	100		
	F	00	00	01	04	05	15	25	42	63	66	71	77	82	82	84		
8	M	01	03	09	16	26	37	58	71	86	99	104	106	108	109	109	110	110
	F	00	00	00	01	01	01	01	01	01	06	09	23	45	65	88	100	128
9	M	15	15	15	22	22	25	25	26	26								
	F	01	01	01	05	08	23	43	56	59								
10	M	21	40	51	51	59	59	59	59	59	59	59	59	60	60	60		
	F	00	00	00	01	02	04	06	11	26	34	45	54	61	74	79		
11	M	20	22	26	26	32	33	38	40	44	47	47	51	61	65	68	68	69
	F	00	00	00	00	00	00	00	00	00	00	00	01	07	11	30	42	82
12	M	47	59	65	73	75	75	76										
	F	04	07	26	46	64	83	88										
13	M	17	33	47	58	58	60	60	60	60								
	F	00	00	01	11	14	32	56	63	78								
14	M	31	32	32	32													
	F	05	18	46	63													
15	M	02	08	20	26	38	43	53	60	61	66	71	72	73	74	75	75	
	F	00	00	00	00	00	00	01	07	13	18	34	43	55	67	77	84	

16	M	01	10	21	33	34	35	35	36	36	36	36	36	37				
	F	00	01	03	03	03	23	34	39	73	79	80	82	83				
17	M	33	43	43	44	44	44	44										
	F	01	05	05	05	05	07	10										
18	M	05	13	25	26	27	28	28	28									
	F	00	00	10	14	25	36	49	58									
19	M	28	82	87	88	90	90	92	92									
	F	00	11	17	37	54	64	71	79									
20	M	15	15	18	19	20	20											
	F	02	05	26	34	49	51											

***Note that each serial number in the table represents all eggs laid by a single mosquito at a particular time (i.e. a single egg-raft). The data is a cumulative frequency of the emerging mosquito.

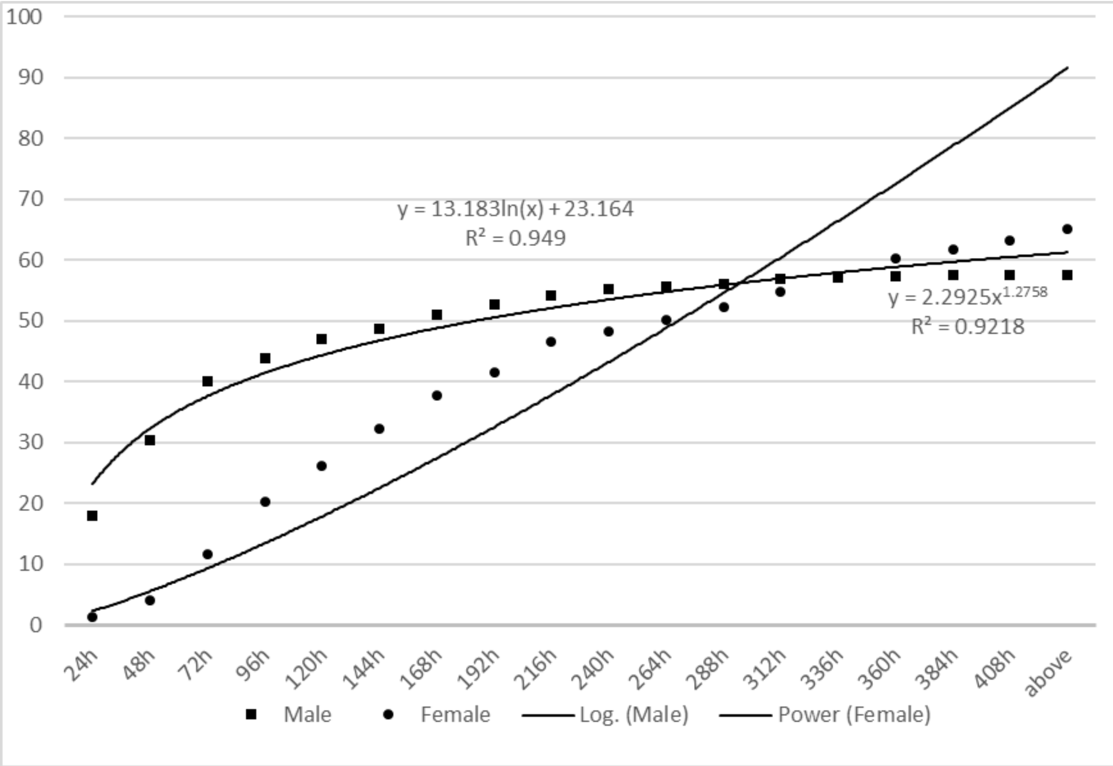

Fig 1: Scatter-plot showing the Trend Followed by emerging Male and Female *Culex spp*

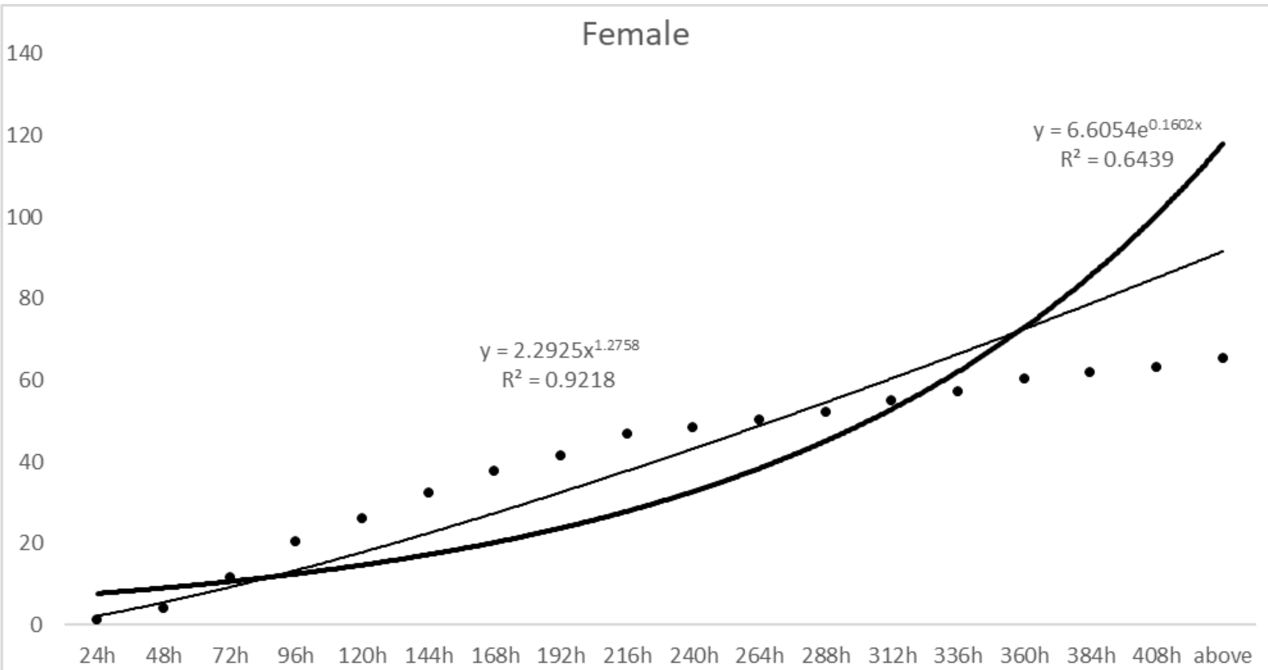

Fig 2: Comparative Analysis (for best fit) of two Trends on Female Scatter-plot

4. Discussion

Mosquitoes of the genus *Culex* were used in the study of emergence pattern because their eggs are laid in natural clusters (egg-rafts), thereby making it easy to sample and observe all offspring of a single parent produced at a particular time. When observed in a laboratory environment, emerging mosquitoes were found to shed their pupal skin to become adults in a manner that is not random but follows a well-defined pattern. This pattern depends on the sex of the emerging adult.

4.1 Pattern of Emergence in Males

The trend followed by emerging males could be described as a logarithmic function of the emergence time at 24 hours interval. A logarithmic function with base 'b' where b>0 and b≠1, is denoted by Log_b and is defined by Y= Log_b x if and only if b^y= x [4]. The logarithm to base 'e' is an important function also known as the natural logarithm. In this case 'e' is approximately 2.718 and an alternative notation ln(x) is often used instead of Log_e x [5].

From our model of male emergence (refer to fig.1), 'Y' represents the number of mosquito that has emerged while 'x' is the emergence time at 24 hours interval. The numbers 13.183, placed before ln(x), and 23.164, placed after the positive sign, in the equation for male emergence are constants. The claim that male emergence is logarithmic in nature is supported by the fact that the trend-line reliability coefficient (R^2) shows a 95 percent common variation between the line and the male scatter-plot. Errors in measurement could account for the remaining 5 percent.

4.2 Pattern of Emergence in Females

In an attempt to look for goodness-of-fit in female emergence, the exponential function model was compared with power regression model (refer to fig.2). This was done for two reasons. Firstly, logarithmic functions and exponential functions are inverse operations [5]. Another reason is, graphically, power functions can resemble exponential or logarithmic functions for some values of 'x'. However, as 'x' gets very large the functions begin to diverge from one another [6].

The trend followed by emerging female mosquitoes could best be described as a power function (or power regression) of the emergence time at 24 hours interval since the reliability coefficient, R^2, shows a 92 percent common variation between the line and the female data; contrary to the exponential model which shows a 64 percent common variation with the same data.

It is worthy of note that a power function is a mathematical function of the form, $Y = ax^p$; where a≠0 is a constant and p is a real number [6]. The meanings of 'x' and 'Y' remain the same.

4.3 Hypothesis on Why a Pattern Exist

Since all eggs laid by a single parent at a particular time were observed to hatch at the same time, there may be a sex-based difference in the rate of growth and development of the immature stages. If such a difference is real, then the immature males develop faster than the immature females of the Species.

4.4 Insight into the Consequences of Patterned Emergence

The pattern creates a loop between the emerging male and female mosquitoes (see fig.3). This means that at any point in time there is an unequal distribution in the number of males and females offspring from the same parent that are emerging. If we make the assumption that mating occurs only between mosquitoes that emerge within every 24 hour time interval; and also that males mate only once in their life time, then this unequal distribution will limit the frequency of successful mating that may occur between offspring of same parent produced at the same time. However, in a complex breeding environment, which is characterized by many eggs produced by different parents at different time, there will be a higher frequency of successful mating between offspring of different parents that are in alternate phase of emergence. From our illustration (see fig.3), the period when there is rapid emergence of female produced by a first parent (F1) will correspond with the period when male produced by a second parent (M2) begins to emerge. Mating between offspring of different parents may promote genetic diversity within the species.

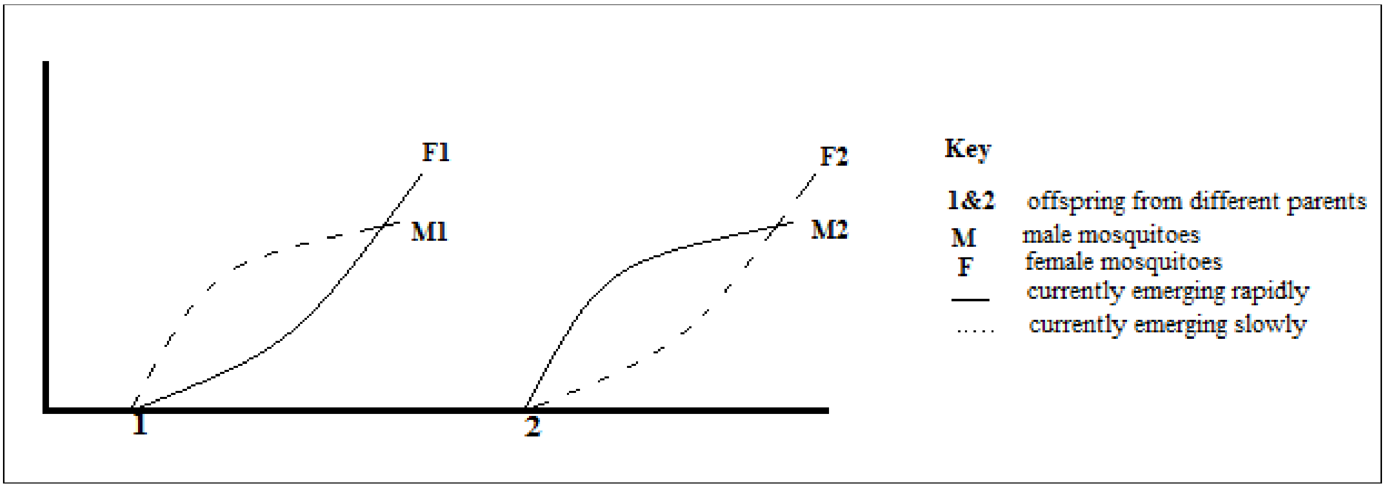

Fig 3: Patterned Emergence in a Complex Environment

5. Conclusion

Emergence in *Culex spp* is an event that does not occur in a random manner, but follows a well-defined pattern. When observed in the laboratory, the species was found to emerge in a pattern that depends on their sex. Emerging males follow a pattern that could be described as a logarithmic function of the emergence time at 24hrs interval. The pattern followed by emerging females could best be described as a power function (or power regression) of the emergence time when compared with the exponential function both at 24hrs interval. A difference in the rate of growth and development which may exist between the male and female immature stages of the mosquito is a probable cause of the pattern. As a consequence, the pattern reduces the number of successful mating that may occur between offspring of same parent hatched at same time. However in a complex breeding environment, the pattern may promote genetic diversity.

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