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Effects of Temperature on the Growth and Development of Culex pipiens Complex Mosquitoes (Diptera: Culicidae)

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Abstract: *This study sought to establish the direct effects of varying temperatures on the growth and development of the two members of the domestic mosquito, Culex pipiens complex; Culex pipiens pallens* Coquillett *and Culex pipiens molestus* Forskal. *The methods used were similar to those used by [18]. The mosquitoes used were obtained from colonies reared at the Kosin University; south Korea, Insectary at a temperature and relative humidity regime of 27 ± 1 °C and 75 ± 5% RH, respectively and a 13:11 light and dark photoperiod. The effects were evaluated in terms of embryonation times, length of the larval and pupal stages, the survival rates and maximum longevity of the female. Varying temperatures were found to have effects on egg embryonation, the lengths of the pupal and larval periods and the survival and longevity of the female mosquitoes. The results showed that in lower temperatures embryonation took longer among the 20°C and 24°C the larval and pupal stages were longer and the female mosquitoes lived longer. In higher temperatures (28°C), embryonation was faster, the larval and pupal stages were shorter and the females did not live for as long as they did at 20°C and 24°C. There were no significant differences (p > 0.05) in egg embryonation times between the two subspecies, at the three temperatures used in the study. There were no significant differences in the larval period between the two subspecies between 24°C and 28°C. However, the length of the pupal period in the two subspecies differed significantly (p < 0.05), at the three experimental temperatures. The pupal period of Cx. Pipiens pallens averaged 5.5, 6.5 and 2.7 days while that of Cx. pipiens molestus averaged 6.4, 4.3 and 2.28 days at 20°C, 24°C and 28°C temperatures, respectively.*

Key word*: Culex pipiens, Culex molestus, Embryonation, longevity, survival*

I. Introduction

Weather can influence physiological events in insect populations by modifying the activity of the endocrine system, which then influences survival, development and reproduction [1]. The study provided information on the direct influence of temperature on the development of eggs, larvae, and pupae of two species of *Culex pipiens pipiens* and *Culex* pipiens molestus, members of *Culex pipiens* complex. Mosquitoes are known to have slow development but live long in low temperatures, fast development in summer and in certain cases stop growth and development and go into diapause when the temperatures become unfavorable [2].

Many studies on the effects of temperature on mosquitoes have tended to concentrate on the study of diapause, freezing tolerance and mosquito ecology with a few studies focusing on the direct effects of temperatures on the active stages of the mosquitoes' life cycle [1]. Temperature effects on growth and development vary greatly on the different zoogeographical regions of mosquito distribution with temperature being favorable throughout the year in the Afro-tropical zone. In the cool temperate zone, breeding and disease transmission are limited to the warm spring and the hot summer [3].

As the human population in the tropical and subtropical regions of the world continue to increase, large portions of the tropical forests are being cleared for settlement and farming. For industrial and technological advancement, the developed countries cause huge increases in the burning of fossil fuels. The total results of the ongoing change in the poor and developed countries are the huge additions of greenhouse gases like methane, carbon dioxide and nitrogen oxide into the earth's, atmosphere [4]. Global temperatures are likely to rise as predicated due to global warming caused by human activities on the earth's surface [5]. This rise in temperature could have the effect of increasing the range and land surface over which mosquitoes can survive with the overall result of expanding the range of transmission of mosquito borne diseases [6].

Increased temperatures are also suspected to be associated with greater desiccation which could cause higher mortality of the eggs, larvae and adult mosquitoes [7; 8; 9; 10]. Temperatures may affect egg viability

and subsequent embryonation [11], larval development [12], blood feeding behaviour [13], female fecundity [14], survival and longevity [15; 16]. This would have a total effect on the population dynamics of the mosquitoes. For example, mosquitoes reared at high temperature tend to develop faster but also tend to be smaller in body size [16; 12] and this is important because the female size is related positively to fecundity [16].

Experimentation on the effects of rearing temperatures on mortality, developmental rates and female adult sizes and survival rates is important as it helps understand the relationship between varying ambient temperatures and population dynamics of the mosquito species[12], The information helps to predict how regional differences in temperatures, seasonal differences within the same area and possible global warming due to climate change that can affect the range of mosquito expansion and disease transmission. The area of existence of dengue virus and its vector; *Aedes aegypti* and *Aedes albopictus* have extended to relatively formerly colder areas of Asia and North America creating a potential for outbreak of dengue fever in these new regions [17].The distribution and performance of the members of the *Cx. pipiens* complex are influenced by the ambient temperature as shown by the results of this study.

II. Materials And Methods

Experimental Mosquitoes

The methods used were similar to those used by [18]. The two members of the domestic mosquito, *Culex pipiens* complex; *Culex pipiens pallens* Coquillett and *Culex pipiens molestus* Forskal used in the tests were obtained from colonies reared at the Kosin University Insectary for several years now. The mosquitoes were reared and maintained at a temperature and relative humidity regime of 27 ± 1 ^{o}C and 75 ± 5% RH, respectively and a 13:11 light and dark photoperiod. The larvae were fed on a mixture of laboratory chow and brewer's yeast at a ratio of 2:1. The adults were fed and maintained on 10% sucrose solution presented using methods described by [19]. The mosquitoes were reared and maintained free from insecticides and repellents.

The performance of *Cx. Pipiens pallens* and *Cx. Pipiens molestus* was evaluated under three different temperature regimes. The tests were carried out in growth chambers/incubators (Model MIR 152, Sanyo Electronic Co, Tokyo, Japan) set at 20^{o}C, 24^{o}C and 28^{o}C and daily light and dark cycles of 12:12 hours to simulate the fall, spring and summer conditions. The humidity levels inside the growth chambers were monitored by placing a battery-powered temperature and humidity digital meter inside each growth chamber. This was necessary because like temperature, humidity influences growth and development in mosquitoes and also modifies the effects of temperatures. The performance of the two *Culex* species was evaluated in terms of their embryonation period, larval and pupal periods in three temperatures. Twelve hours before the start of the experimentation, the three growth chambers were set at their respective temperature of 20^{o}C, 24^{o}C and 28^{o}C to ensure they were in good working condition and to preset the temperature before inserting the eggs.

Tests for embryonation periods

The newly laid eggs, white in colour, were collected from the respective adult mosquito species for setting up the experiments in the morning. For embryonation, the fresh eggs were placed in the eighteen 2,000 ml clear/colorless hard plastic hatching containers. The18 containers were divided into three groups of 6 containers, to go into each of the growth chambers. Each growth chamber held three containers of *Cx. Pipiens pallens* and three of *Cx. Pipiens molestus* eggs at the specified temperature. A single raft of eggs (approximate 100-150 eggs) was placed in each of the 6 egg-hatching plastic containers with about 1,000 ml of dechlorinated water (Fig. 6.1). Each of the 18 egg-hatching containers was labeled with the full name of the species, the temperature at which the test was set, and the date of the starting of the test and given an identifying number (e.g. *Cx. Pipiens pallens* 2009/June/1st, 20^{o}C, Container 1, 2 or 3).Observations were made at intervals of six hours and the numbers of larvae hatching from the eggs were recorded. The recording was made on a prepared data entry form and each test was replicated three times.

A record of the number of larvae hatching was taken until maximum hatching was attained, and this was indicated by lack of further increase in larval numbers in each of the hatching containers. The embryonation period was calculated by multiplying the number of larvae by the number of days taken to hatch and then divided by the maximum number of larvae hatched for that replicate. This was done for all the hatchings prior to the maximum hatching and added to give the total hatching period in days.

For example, if replicate 1 at 20^{o}C for *Cx. Pipiens pallens* hatched 59 larvae in 2.5 days, to get the embryonation period, (2.5 x 59)/59 to give 2.5 days as the embryonation period. If replicate 2 of the same species at the same temperature hatched 40 larvae after 2 days and then after 2.5 the number increased to 47, 47 is the maximum hatching. To get the embryonation period, (2.0 x40)/47 + (2.5 x47)/47=2.25(Same as taking the average of the two replicates) is the embryonation period in days. These calculations were repeated with all the

replicates at the three temperatures at which the experimentation was done. The average embryonation period was obtained by adding up the total for the three replicates and dividing by three. The embryonation periods were statistically analyzed using t-tests and ANOVAR at p=0.05 using SPSS 12.0 program (SPSS Inc. 2005) for Windows, to find out if there were significant differences in embryonation period between the two species and within the same species at the three temperatures used in the tests.

Fig1 The arrangement of egg-hatching containers in the growth chamber for development under controlled temperature with aeration system shown

Tests for the length of the larval period

Once hatching had taken place, aeration system was put in place to stop scumming and frothing of the larvae rearing water (Fig.6.1). The larvae were fed with a mixture of laboratory chow and yeast at a ratio of 2:1, respectively.

Observations made at six hour intervals and the numbers of pupae forming in each of the egg-hatching container were recorded. The larval period was calculated by multiplying the number of pupae at each observation point by the number of days since the larvae were hatched. For example, if at 14.5 days after larvae hatching, 6 pupae had formed the total period is 14.5 x 6= 87, this was done for all the observations, totaled up and divided by the total number of pupae from that particular replicate (container). For example, if in replicate 2 at 20^{o}C a total of 39 pupae formed in a cumulative duration of 608.5 days, the actual larval period for each larva was obtained by 608.5/39= 15.6 days. This means each larva at 20^{o}C lived an average of 15.6 days before pupation set in. This was done for all the replicates at three temperatures. An average larval period at each temperature was calculated by adding up the totals for the three replicates and dividing by three. The larval periods were statistically analyzed using t-tests and ANOVAR at p=0.05 using SPSS 12.0 program (SPSS Inc. 2005) for Windows to find out if there were significant differences in the larval periods between the two species and within the same species at the three temperatures used in the tests.

Tests for the length of the pupal period

The pupae were moved from the breeding water into beakers within the same growth chamber. Eighteen 2,000 ml and eighteen 10 ml glass beakers were used for the tests. They were divided into three groups of twelve beakers per chamber (six 2,000 ml and six 10 ml beakers). The 12 beakers in each chamber were then divided in two groups each of three 2,000ml and three 10 ml beakers, one group of beakers to hold pupae of *Cx. Pipiens pallens* and the other pupae of *Cx. Pipiens molestus*. The 10 ml beaker with about 5.0 ml of clean water was placed inside the 2,000ml beaker. The 2,000ml beakers were then tightly and covered with a piece of cotton cloth and a small slit made in the middle to allow for insertion of an aspirator into the beaker. A ball of cotton wool was used to close the slit to prevent adults from escaping out of the beaker once eclosion occurred. The beaker was then labeled with the name of the species, date of the test, temperature of the chamber, a number corresponding to that of the hatching-container from which the pupae were being transferred and the number of pupae placed therein (e.g. *Cx. Pipiens pallens* 2009/June/1st, 20^{o}C, beaker 1, 2 or 3, 159 pupae). The pupae were collected with a little water using a large pipette and moved into the 10ml beaker inside the 2,000 ml beaker and then placed inside the growth chamber at the correct test temperature. To feed the emerging adults, the feeding cotton swab used to close the slit was irrigated with 10% sugar water and covered with clear para-film to prevent evaporation and drying up.

Observations were made at intervals of six hours and the number of adults emerging recorded until all the pupae underwent eclosion. The length of the pupal period was obtained by adding up the total numbers of adults emerged, in each replicate divided by the total period in days in which all the adults emerged. For example, if at 20^{o}C on day 20, 3.5 days after the pupae were placed in the beaker only 1 adult had emerged the total pupal period is given by 3.5 x 1=3.5 days. This was done for each observation period through the days until no more adults emerged. For example, if a total of 150 adults emerged over an accumulative period of 75 days, the actual pupal period was obtained by 150/75=2.0 days, meaning it took an average 2.0 days for each larva at 20^{o}C to emerge into an adult. This was calculated for all the three replicates at each of the three temperatures. An average for each temperature was obtained by adding up the pupal periods for the three replicates and dividing by three. The pupal periods were statistically analyzed using t-tests and ANOVAR at p=0.05 using SPSS 12.0 program (SPSS Inc. 2005) for Windows to find out if there were significant differences in the pupal periods between the two species and within the same species at the three temperatures used in the tests.

Tests on survival and maximum longevity of *Culex pipiens* female mosquitoes

After the adults emerged, a new set of experiments was initiated at each temperature to compare survival rates and maximum longevity of the females of *Cx. Pipiens pallens* and *Cx. Pipiens molestus* at the three temperatures of 20^{o}C, 24^{o}C and 28^{o}C. Six 500ml beakers were used in each growth chamber. The beakers top was tightly covered with a piece of cotton material with a small slit made at the middle to allow for the insertion of the aspirator to place the females into the beaker. A feeding swab irrigated with 10% sugar water was placed over the slit at the top of the beaker and covered with clear Para film to prevent drying up due to evaporation. Each beaker was labeled with the species name, the date, the temperature and number of adults introduced inside. Females were transferred from the 2,000 ml beakers with an aspirator to the 500ml beakers. The beakers were then placed into the growth chambers. Each test was replicated three times.

Observations were made at intervals of 12 hours and the number of dead and live females recorded. These observations were continued for the three temperatures until all the females in all the replicates died. To work out the survival period for the females, the point at which the females died was considered as indicating the number of days they have lived since emergence from pupae. For example if at 28^{o}C, in replicate 2, three females died at day 2, they were considered to have lived for a total of 2 x 3= 6 days. This was calculated for all the days until all the females died. If in replicate 2 of the test at 28^{o}C, had 78 females all dying after an accumulative period of 633 days, average survival in days is given by 663/78= 8.1 days. This means that each female in the replicate lived an average of 8.1 days then died. This was calculated for all the replicates in the three temperatures. An average for each temperature was calculated by adding the survival number of days for the three replicates and then dividing by three. It was taken as longest period in days over which the females in each replicate survived. Maximum longevity was calculated by taking the point of death of the longest surviving females in each replicate and working out an average for the three replicates at each temperature.

Statistical Analysis

The survival and longevity data were statistically analyzed using t-tests and ANOVAR at p=0.05 using SPSS 12.0 program (SPSS Inc. 2005) for Windows to find out if there were significant differences in the survival periods between the two species and within the same species at the three temperatures used in the tests.

III. Results

The results showed that temperature has effects on embryonation, larval and pupal periods as well as the length of survival time in mosquitoes. The results of embryonation periods, larval and pupal period, survival and maximum longevity are given in Tables 1 to 5 below.

Table1. Comparison of embryonation periods between *Culex pipiens pallens* and *Culex pipiens molestus* eggs under three different temperatures

	Mean day ± S.D	
Temp(^{o}C)		
	Cx. pipiens pallens	*Cx. pipiens molestus*
20	2.19 ± 0.27a*A**	2.18 ± 0.28aA
24	1.60 ± 0.23 aA	1.50 ± 0.00aB
28	1.12 ± 0.21aB	1.16 ± 0.28aB

*means in the same row followed by the same lower case letter are not significantly different At the 5% level of probability.

**means in the same column followed by the same upper case letter are not significantly different at the 5% level of probability.

There were no significant differences in the embryonation periods between the two species under each of the three experimental temperatures at $p > 0.05$. At 20^{o}C, the eggs of *Cx. Pipiens pallens* took an average of 2.19 days for embryonation while those of *Cx. Pipiens pallens* took 2.18 days. At 24^{o}C, the eggs of the *Cx. Pipiens pallens* and *Cx. Pipiens molestus* took an average of 1.6 and 1.5 days, respectively. At 28^{o}C, the eggs of the two species of *Cx. pallens* and *Cx. molestus* took 1.1 and 1.16 days, respectively (Table 1).

Within each species, there while no significant differences ($p > 0.05$) in embryonation period between 20^{o}C and 24^{o}C in *Cx. Pallens pipiens* but there were significant differences between the embryonation period at 20^{o}C and 24^{o}C, and that of 28^{o}C ($p < 0.05$). In *Cx. Pipiens molestus*, there were no significant difference in the embryonation period between 28^{o}C and 24^{o}C but the embryonation periods at the two temperatures significantly differed with that at 20^{o}C (Table 1).

Table 2. Comparison of the length of the larval period in *Culex pipiens pallens* and *Culex pipiens molestus* under three different temperatures

	Mean day ± S.D	
Temperature (^{o}C)	*Culex pipiens pallens*	*Culex pipiens molestus*
20	16.35 ± 2.20a*A**	17.70 ± 0.60aA
24	9.50 ± 0.70bB	13.60 ± 0.60aB

28	7.90 ± 0.85aB	9.40 ± 0.40aC

*Means in the same row followed by the same lower case letter are not significantly different at 5 % level of probability.
**Means in the same column followed by the same upper case letter are not significantly different at 5 % level of probability.

Temperature was found to affect the length of the larval period. There were no significant differences in the larval period between the two species at 20°C with the larvae of *Cx. Pipiens pallens* living for an average of 16.35 days before undergoing pupation, while those of *Cx. Pipiens molestus* took an average 17.70days. At 24°C, the larval period for the two species differed significantly with the larvae of *Cx. Pipiens pallens* taking an average of 9.5 days before pupation, whiles those of *Cx. Pipiens molestus* took an average of 13.6 days (Table 2). There were no significant differences in the length of the larval stage at 28°C for the species. Within the *Cx. Pipiens pallens*, there were significant differences in the larval periods between 20°C and 24°C but there were no significant difference between 24°C and 28°C. The length of the larval period differed significantly between 20°C and 28°C. Within the *Cx. Pipiens molestus*, the length of the larval period differed significantly at all the three temperatures.

Table 3. Comparison of the length of the pupal period in *Culex pipiens pallens* And *Culex pipiens molestus* at three different temperatures

Temperature (°C)	Mean day ±S.D *Culex pipiens pallens*	*Culex pipiens molestus*
20	5.54 ± 0.25b*A**	6.40 ± 0.40aA
24	6.53 ± 0.60aA	4.34 ± 0.32bB
28	2.73 ± 0.20bB	2.28 ± 0.67aB

*Means in the same row followed by the same lower case letter are not significantly different at the 5 % level of probability

**Means in the same column followed by the same upper case letter are not significant different at the 5 % level of probability

The pupal period seems to have more influence from temperatures. The length of the larval period differed significantly between the two species at all the three temperatures at $p < 0.05$ (Table 3). Within the individual species, there were no significant differences in the length of the pupal at 20°C and 24°C but the two differed significantly with the pupal period at 28°C in *Cx. Pipiens pallens*. In *Cx. Pipiens molestus*, there were no significant differences between the pupal periods at 28°C and 24°C ($p > 0.05$) but the two differed significantly with the pupal period at 20°C ($p < 0.05$).

Table4. Survival in days of females of two of *Culex pipiens* complex species at 3 different temperature regimes

Temperature (°C)	Mean day ± S.D *Cx. pipiens pallens*	*Cx. pipiens molestus*
20	18.9 ± 1.6aA	29.2 ± 9.3aA

24	13.5 ± 4.1aA	9.7 ± 2.2aB
28	7.7 ± 1.1aB	8.6 ± 0.7aB

*Means in the same row followed by the same lower case letter are not significantly different at the 5 % level of probability.

**Means in the same column followed by the upper case letter are not significantly different at the 5 % level of probability.

There were no significant differences in the survival rates between the two species at all three temperatures (p> 0.0. Within the species there were no significant differences in the survival of the *Cx. Pipiens pallens* at 20°C and 24°C but the two differed significantly with the survival at 28°C. In the *Cx. Pipiens molestus* the survival differed significantly between 20°C and 24°C but it did not differ significantly between 24°C and 28°C (Table 5and Figs. 2, 3 &4).

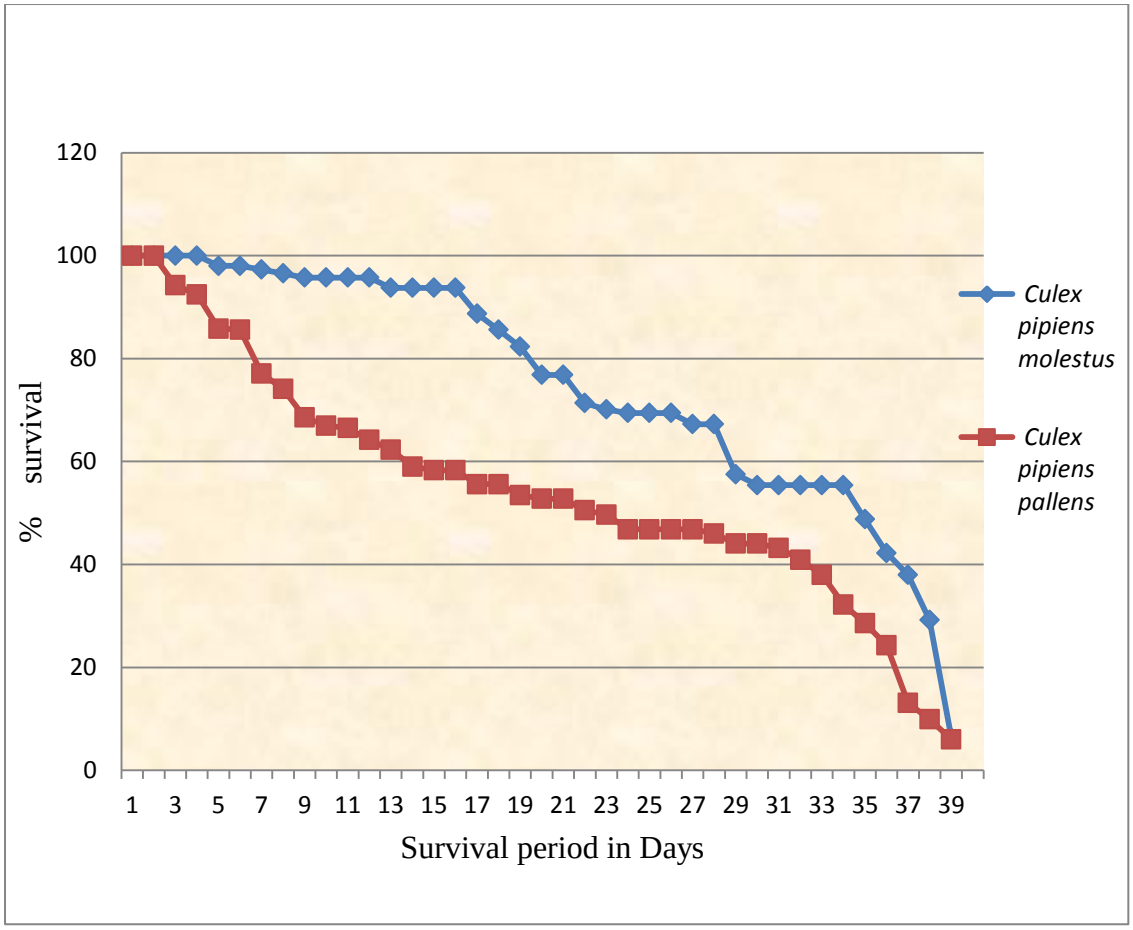

Fig 2 Comparison of survival rates of two species members of *Culex pipiens* complex at 20°C.

The survival in the females of the two *Cx. pipiens* species was inversely proportional to the temperature. More females survived in the lower temperatures than those at higher temperatures while fewer females survived in high temperatures.

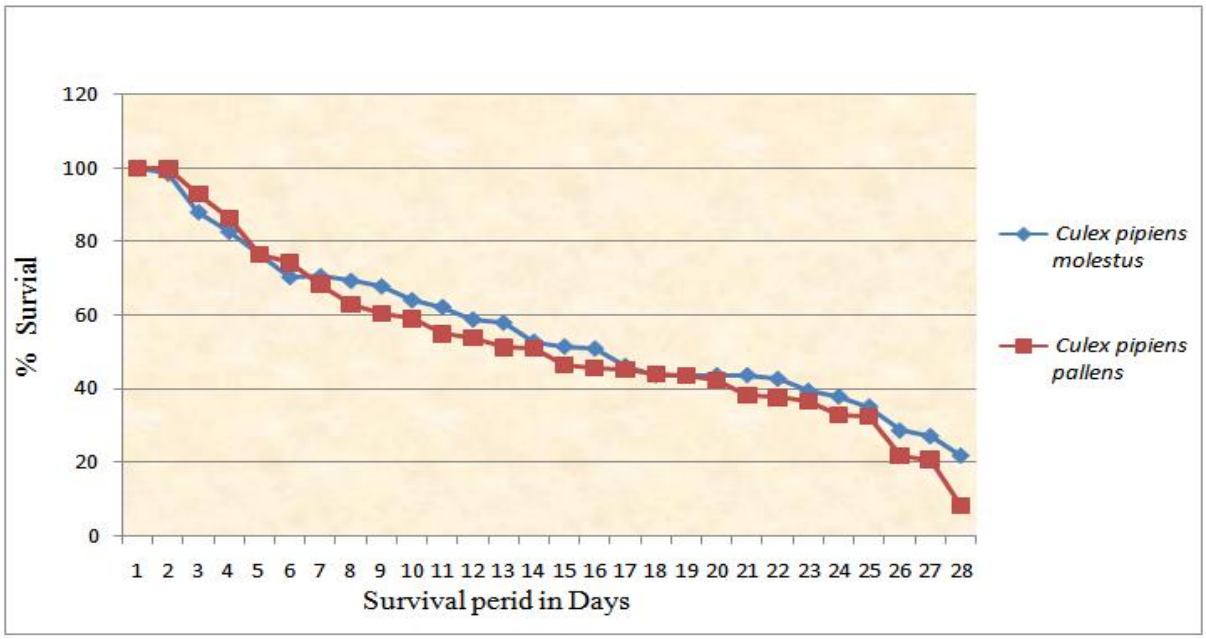

Fig 3 Comparison of survival rates of two species members of *Culex pipiens* complex at 24°C.

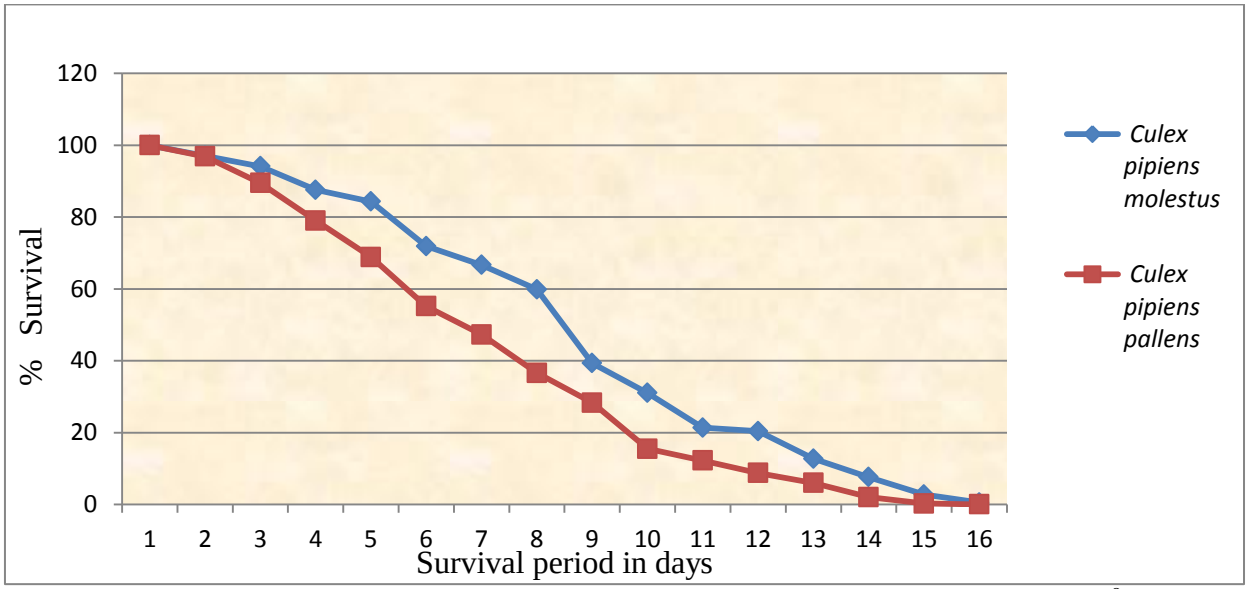

Fig. 4. Comparison of survival rates of two species members of *Culex pipiens* complex at 28°C.

Table5. Maximum longevity in days by female mosquitoes of two species of *Culex pipiens* complex

	Max longevity in days ± S.D	
Temp (°C)	*Culex pipiens pallens*	*Culex pipiens molestus*
20	37.3 ± 0.6aA	38.3 ± 0.6aA
24	26.3 ± 0.6aB	27.3 ± 0.6aB
28	14.3 ± 0.6a*C**	15.3 ± 0.6aC

*Means in the same row followed by the same lower case letter are not different significantly at the 5 % level of probability.

**Means in the same column followed by the same upper case letter are not

significantly different at the 5 % of probability

The maximum longevity was inversely proportional to the temperatures; as the temperature increases the females of both species of *Cx. pipiens* tended to live for shorter periods. The longest living females were those reared at 20^{o}C and the shortest living those reared at 28^{o}C.

The shortest periods of immature development were observed at 28^{o}C with averages of 7.9 ± 0.9 days (*Cx. pipiens pallens*) and 9.4 days (*Cx. Pipiens molestus*), while the longest periods were at 20^{o}C with averages of 16.4 days (*Cx. pipiens pallens*) and 17.7 days (*Cx. pipiens molestus*). There were significant differences ($p < 0.05$) in the length of both the larval and pupae periods at the three temperatures in each subspecies. However, there were no significant differences ($p > 0.05$) in the length of the larval period between *Cx. pipiens pallens* and *Cx. pipiens molestus* at 20^{o}C and 28^{o}C although these were significantly different at 24^{o}C. The length of the pupal stage differed significantly ($p < 0.05$) between the two subspecies. These results indicate the ability of the members of the*Cx. pipiens* complex to survive over wide range of temperatures.

IV. Discussion

From the results, it was evident that temperatures influence egg embryonation, the length of the larval and pupal stages of the mosquito as well as their survival and maximum longevity of the two species *of Cx. pipiens. Temperatures have been shown to affect both the biology and the ecology of the Cx. Pipiens* complex *[20]. The results of this study seem to fall in agreement that at higher temperatures, the mosq*uitoes' performance in terms of embryonation, larval and pupal growth is faster yet the mosquitoes generally live for a shorter period. At low temperatures, the mosquitoes took long to go through their life cycle and lived for long. In many parts of the world, the transmission of mosquito borne diseases is seasonal and is linked to rainfall and temperature patterns. Temperature is a key determinant of boundaries of disease transmission and distribution. This is achieved either by limiting the distribution of the vectors or because below certain temperature the pathogen cannot complete its life cycle within the vector. Temperatures have effects on the emergence, survival and the subsequent behavior of the adult *Cx. pipiens* mosquitoes [21]. This agrees with the findings of our study where in higher temperatures the mosquitoes complete their life cycle faster but also died earlier with the females living for an average of 7.7-8.6 days at 28^{o}C.

Higher temperatures are known to shorten the extrinsic incubation period of pathogens within their mosquito vectors [22]. The rearing temperatures influence the vector competence of the mosquitoes [23] as vector's competence tends to be depressed by decreasing the temperature for adult mosquitoes. In summer when temperatures are high within the tropics, the water temperatures in the containers where the domestic mosquitoes like *Cx. Pipiens* complex breed tend to increase. This increase as long as it is tolerable, tends to favour their role as disease vectors but certain temperature rises may go way beyond the levels at which the mosquito immature stages can survive [18]. When the larvae of *Aedes aegypti* were exposed to a range of varying temperatures, mortality was observed to increase with increase with temperatures above certain levels with the temperature being completely lethal at 43^{o}C [18]. Adults reared at higher temperatures tend to be more susceptible to pathogens such as arbovirus and temperature has been thought to act as a selection factor for the mosquitoes responsible for the transmission of diseases like Chikungunya virus.

High temperatures induce formation of heat shock proteins in certain mosquitoes such as *Anopheles* which have been found to contribute to their success as disease pathogen vectors [24]. Similar heat shock proteins induced by rearing mosquitoes under high temperatures have been observed in *Ae. aegypti* and *Ae. albopictus* [25]. In tropical regions, where temperatures are often high, the mosquitoes breed in water containers whose water temperature rearing goes beyond the 41^{o}C [18] but supports the disease transmission by mosquitoes in the region. An increase in temperature above the average in an area, where mosquito borne diseases are endemic, could result in a number of events. It could enhance the selection of temperature-tolerant mosquitoes within the mosquitoes' population with a long longevity. It could also reduce the intrinsic incubation period of the pathogen within the vector mosquitoes which could result in epidemic outbreaks. It could also cause increased susceptibility of the mosquitoes to the pathogens like virus hence increasing their vector competence [26]. Studies using *Ochlerotatus* a*lbifasciatus* mosquitoes showed that the life cycle in these mosquitoes is directly influenced by the ambient temperatures. The length of its life cycle varied from a minimum of 6 days at 24^{o}C to a maximum of 32 days at 13^{o}C [27].

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