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Comparative Assessment of Immature Survivorship and Developmental Duration of Culex Pipiens Pipiens (Diptera: Culicidae) Mosquito Vector Populations in North Central Nigeria

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Abstract

Background

Culex mosquito vectors of human diseases have received relatively lesser attention from mosquito biologists even though the diseases transmitted by these mosquitoes rank foremost among public health challenges worldwide. As a result, very little is known about spatial ecology of these mosquitoes, especially, the dynamics of their larval development; an important factor that governs the success of larviciding measures. This need informed the present study to elucidate spatial variations in survival and developmental rates of *Culex pipiens pipiens* mosquito populations in North Central Nigeria.

Methods

Day old larvae of *Culex pipiens pipiens* mosquitoes were collected from four widely-spaced localities in the area namely; Gidan Kwano (A), Bosso (B), Maikunkele (C) and Chanchaga (D). The larvae were reared under laboratory conditions and monitored for immature life-stage survival and developmental rates, following standard procedures.

Results

The results indicated that Total Immature Survival Rate (TISR) was very high (>88%), though varied significantly (P<0.05); ranging from 88.67±7.58% in site C to 95.08±1.68% in site D. While, Pupal Stage (PS) survivorship varied significantly (range = 86.92±12.16 to 97.24±2.48%), those of Total Larval Stage (TLS) (Range = 86.76±7.99% to 93.39±4.48%) were insignificantly different (P>0.05) among the sites. The fastest developing immature population of *Cx. p. pipiens* in the area was that from site C, which took 8.67±2.03 days. This value was significantly lower than those from other sites; taking as long as 10.10±0.94% days in site B.

Conclusion

These results revealed significant spatial variation in survival and developmental rates of immature *Cx. p. pipiens* populations in north central Nigeria; and such variations appear to be driven by endogenous factors. This spatial heterogeneity may influence the vectorial importance and ecological adaptations of the species in the area, and therefore demand site-specific larval vector control strategies and intervals in the area.

Introduction

Three major mosquito *Genera* namely *Anopheles, Aedes,* and *Culex* are involved in the transmission of human diseases including, malaria, yellow fever, filariasis and dengue fever (El-Badzry and Al-Ali, 2010; Balakrishnan *et al*., 2011; Paulraj *et al*., 2011). However, emphasis has always been placed the *Anopheles* and to some extent *Aedes* (Njan-Nloga *et al*., 2007; Olayemi *et al*., 2011a; Olayemi *et al*., 2012), simply because diseases transmitted by theses mosquitoes (i.e. malaria and yellow fever) are perceived to be more widespread and affect more people than those transmitted by *Culex* such as filariasis. To this end, very little is known about the ecology of *Culex* mosquitoes in Africa in general, and Nigeria in particular. Yet diseases transmitted by *Culex* mosquitoes have been reported all over the Continent (Anosike *et al*., 2005) and country (Anosike *et al.,* 2003; Braise *et al.,* 2003; Awolola *et al.,*, 2004; Terranella *et al.,* 2006; Ibanga *et al.,* 2008; Obi *et al.,* 2010; Omudu and Okafor, 2010).

However, Studies have shown that the biology and ecology of mosquitoes often vary spatially (Collins and Paskewitz, 1995; Onyabe and Conn, 2001), with serious implications for disease transmission and mosquito vector responses to control strategies (Molyneaux and Gramiccia, 1980; Service, 1985; Olayemi *et al*., 2011b). Therefore, in order to generate baseline information, on the ecological diversity of *Culex pipiens pipiens* mosquitoes in north central Nigeria, this study was carried out to elucidate the spatial heterogeneity in immature survival rate and duration of life-stages of the species in the area.

Methods

Description of the Study Area and Sampling Sites

The study was carried out in and around Minna area of North Central Nigeria. Minna is the capital of Niger state, located within longitude 6^0 33? E and latitude 9^0 27 ? N, covering a land area of 88km^2 with an estimated human population of 1.2 million. Minna enjoys a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20^{o}C, 61.00% and 1334.00mm, respectively. The climate presents two distinct seasons; a rainy season between May and October and a dry season (November - April). The vegetation in the area is typically grass-dominated savannah with scattered trees.

Larvae collection was carried out at four different and widely separated localities: Gidan Kwano (A), Bosso (B), Maikunkele (C) and Chanchaga (Figure 1). Gidan Kwano site is a rural and sparsely populated area, with large expanse of trees and lowland grasses. The site has poor drainage system and the houses are mainly built with mud bricks. The inhabitants are mostly farmers, petty traders and students of the Permanent Campus of Federal University of Technology, Minna. The site is located about 22.km from Minna, the state capital. The Bosso site, on the other hand, is an urban settlement about 7km from Minna. The site is densely populated, with the inhabitants being mostly civil servants and traders. The area hosts the Temporary site of the Federal University of Technology, Minna. The area has very sparse vegetation but enjoys good drainage system. Most of the houses in this site are modern (i.e., built with cement brick and corrugated roofing sheets). Maikunkele site, however, is sparsely populated and located about 16km from Minna. Its inhabitants are mostly farmers with few business people and civil servants. The area has thick vegetation and characterised by poor drainage system.

The Chanchaga Site is densely populated, with good drainage system and sparse grass cover. The area situated about 9km from Minna, hosts the Niger State College of Education and an Army Barracks.

Mosquito Larvae Collection and Identification

Day old larvae were collected between 0700hrs and 0900hrs during the rainy season of 2011. Larval collection was done from conventional mosquito breeding habitats in the four localities, using a 350ml dipper (Azari-Hamidian *et al*., 2011). Collected larvae were transported to the insectary of the Department of Biological Sciences, Federal University of Technology, Minna, for rearing. The larvae were identified using standard taxonomic keys (Hopkins, 1952).

Laboratory Rearing of Larval and Pupal Stages

The study was carried out simultaneously on each of the four sites, with each having four replicates. Each replicate consisted 50 day-old larvae of *Cx. p. pipiens* per bowl. The whole study was repeated immediately after the first exercise, resulting in the monitoring of a total of 1600 larvae from 1st instar stage to adulthood. The larvae were reared following standard techniques (Gerberg, 1970; Das *et al*., 2007; Olayemi and Ande, 2009) in plastic bowls (of 1250 ml capacity) which were labelled appropriately to aid identification, at the rate of 50 larvae/bowl containing 1000ml of water from a borehole. The plastic bowls were inspected every 12 hrs (i.e., 0600 and 1900hrs) each day for dead larvae and pupae, and such were removed and noted. Also, the number of individuals transforming into the next larval stage was noted on daily basis, until pupation.

The larvae were fed with fish feed (Tetramin®), at the rate of 0.32 mg/larva every other day. On every alternate day, the water from the culture bowls was changed, with mortality noted, and larvae in the next instar stage of development noted and transferred to a new bowl; this practice continued till pupation. The pupae were separated daily and placed in plastic bowls (5 cm height and 20cm diameter), half-filled with borehole water. The plastic bowls, with pupae, were labelled appropriately and placed in adult-holding cages for emergence; mortality was noted. Pupae that were unable to emerge or adults that were unable to break free from the pupal case were considered dead. The mean temperature and relative humidity of the insectary, during the study period, were 28.00±1.00^{o}C and 70.20 ± 2.82%, respectively, with 12 L: 12 D photoperiod.

Determination of Immature Stage-Specific Survivorship

Survival rates during the life stages were determined as the proportion of individuals at the beginning of a life stage that successfully entered the next stage, according to the formula of Olayemi and Ande (2009):

$S_i = n_i ?(x_{ni-1}) \times 100$

Where, $n_i$is the numbers of larvae entering instar stage *i,* and $x_{ni-1}$is the number of larva that entered the preceding instar stage.

Determination of Duration of Immature Stages

Duration of immature life stages was determined according to the techniques of Edilo *et al*. (2004) and Olayemi and Ande (2009). Mean larval instar duration and pupal stage duration, in days, were estimated

using the formula:

$D_i = T_i – (t_{i-1})$;

Where, D_i = duration of life stage, T_i = present mean age and t_{i-1} = previous mean age at moulting.

Data Analysis

Statistical analyses of data collected were carried out using SPSS computer software for Windows, Version 15.00 (Install Shield Corporation, Inc.). The mean values of survival and developmental rates of the various immature life-stages of the mosquito species in the 4 localities were compared for statistical significance using ANOVA, at p=0.05 level of significance.

Results

Table 1 shows the immature survivorship rates of different populations of *Culex pipiens pipiens* mosquitoes in north central Nigeria. Generally, Total Immature Survival (TIS) rate was very high (about 90% and above), though varied significantly ($p<0.05$); ranging from 88.67±7.58% in site C to 95.08±1.68% in site D. For the specific immature stages, while pupal survivorship varied significantly (range =86.92±12.16% to 97.24±2.48%), those of Total Larval Stage (TLS) were insignificantly different ($p>0.05$), except for site A (range = 86.76±7.99% to 93.39±4.48%). Again, site C had the least survival rates with respect to TLS and pupal duration.

Survivorship of the larval instar stages was equally very high, as none was less than 80% in all the sites; infact 100% survival was recorded among the L1 larvae of the mosquitoes from site D (Table 1). However, significant ($p<0.05$) variations were still observed in each larval instar stage among the 4 sites. Except for site C, larval instar survival rates were least in the L3 instar in all the sites. On the other hand, the L1 larvae were most successful larval instar in the study sires, except site B where the L2 larvae had the highest survival rate (98.42±2.42%).

The duration of the immature stages of the mosquito species in the 4 sites is highlighted in Table 2. Though, a number of similarities were observed in the patterns of distribution of life-stage survivorship and duration among sites, certain significant differences were apparent. For example, the fastest developing immature population of *Cx.pipiens pipiens* in the area was that from site C, which completed immature development in 8.67±2.03 days. This value was significantly ($p<0.05$) lower than those from other sites; taking as long as 10.10±0.94 days for the mosquitoes from site B to complete such development.

Again, unlike survivorship, duration of pupal stage (range = 1.58±0.62 to 1.95±0.37 days) and L4 larval instar (range = 1.86±0.37 to 2.40±0.42 days) were not significantly ($p>0.05$) different among the sites (Table 2). Total Larval Duration (TLD) (range = 7.04±1.47 to 8.44±0.72 days) had the same pattern of distribution as Total Immature Duration (TID). While, L1 was the shortest larval instar in sites A and D (mean = 1.67±0.16 and 1.20±0.16 days, respectively), the L2 larvae had the shortest in sites B and C (mean = 2.03±0.39 and 1.46±0.20 days, respectively). Except for site D (mean L2 duration = 2.51±0.53 days), the longest larval instar duration (range = 2.21±0.40 to 2.40±0.42 days) was recorded among the L4 individuals in the study area.

Table 1: Stage-specific survivorship of immature stages (%) of *Culex pipiens pipiens* in North central Nigeria during the rainy season of 2011.

See Illustration 1

Table 2: Duration of Immature stages of (days) *Culex pipiens pipiens* in North central Nigeria during the rainy season of 2011

See Illustration 2

Discussion

The results of this study revealed thriving populations of *Culex pipiens pipiens* in Minna area of north central Nigeria, and such mosquito populations have successfully adapted to completing immature development in epidemiologically–important duration. The immature survival and developmental rates of this mosquito species in the area ranged from 88.67±7.58 to 95.08±1.68% and 8.67±2.03 to 10.10±0.94 days, respectively. These results differed considerably from those earlier reported for the species elsewhere. While, Botha *et al.* (1967) reported developmental duration as short as 135.3±4.4 hours (about 5.63 days) in Rangoon, Burma, Hayes and Hsi (1975) observed that *Cx. p. pipiens* mosquitoes took up to 46.8 days to complete immature development. These significant variations in developmental duration of *Cx. p. pipiens* mosquitoes may be due to differences in weather conditions. The existence of thriving populations of *Cx. p. pipiens* mosquitoes in Minna and environs, portends high risk of the transmission of *Culex*-borne diseases such as filariasis, elephantiasis etc., among the human population in the area.

Survivorship of immature stages of this mosquito in the study area was generally high, probably, indicating

high mosquito density, and poses serious threat to public health, in the event of introduction of pathogens of *Culex*-borne diseases into human population. While, pupal survivorship varied significantly among the sites, those of Total Immature Survival rates were insignificantly different. The reverse was, however, the case with respect to life-stage duration, whereby those of Total Larval development varied significantly but the pupal stage lasted for more-or-less the same period. These findings indicate that variation in space significantly influence larval developmental rates of *Cx. p. pipiens* in the area, with the consequent implication of differential turn-over rates of the species in the area. This observation, perhaps, calls for site-specific differential larviciding intervals in different localities of the study area, to ensure that the immature stages get eliminated before adult emergence.

Likewise, spatial heterogeneity in durations of the immature stages of *Cx. p. pipiens* was observed in the area, with attendant implications for mosquito population density and hence, vectorial capacity. Since the mosquito larvae were neither reared on site nor in water media from original breeding habitats, could mean that the heterogeneity in developmental rates, recorded in the area, is controlled by endogenous factors such as genetics. According to Gafur (2004) and Olayemi *et al*. (2011c), most of the biologic and ecologic traits in Mosquito populations are strongly influenced by genetic variability from one locality to another. Therefore, the immature developmental heterogeneity of *Cx. p. pipiens* population in Minna and environs, as revealed by the results of this study, could be an indication of similar heterogeneity in vector competence for pathogen and hence, epidemiological importance of the mosquito populations (WHO, 1975; Olayemi *et al.,* 2009). Confirmed heterogeneity in vectorial attributes of mosquito populations, may also mean significant differences in susceptibility to vector control strategies, that should guide anti-larval interventions.

Though, survivorship of the different larval instar stages were equally very high, significant variations were still recorded within each site. This finding provides a critical information regarding timely targeting of mosquito larviciding interventions at most vulnerable links in the vector's life-cycle, as canvassed by WHO (1975) and Olayemi and Ande (2009). Therefore, in north central Nigeria, aiming larviciding measures to coincide with L3 instar stage of *Cx. p. pipiens,* when mortality was naturally highest among the larvae, may ensure maximum impact.

Conclusion

The findings of this study revealed that significant spatial variation in survival and developmental rates of immature *Cx. p. pipiens* populations exist in north central Nigeria; and such variations appear to be driven by endogenous factors. This spatial heterogeneity will, perhaps, influence the vectorial importance and ecological adaptations of the species in the area, and therefore demand site-specific larval vector control strategies and intervals in the area.

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Illustrations

Illustration 1

Stage-specific survivorship of immature stages (%) of Culex pipiens pipiens in North central Nigeria during the rainy season of 2011.

Larval Collection Site	Larval Instar Stages					Pupal Stage	TIS
	L1	L2		L3			
	L4	TLS					
A	94.25±3.92^{a*}	83.76±9.30^{a}	83.07±12.68^{a}	85.96 ±10.47^{a}	86.76±7.99 a	96.33±7.03^{c}	91.57±5.37^{a} b
B	94.75±6.58^{a}	98.42±2.42^{c}	89.70±11.52^{b}	90.67±10.87 b	93.39±4.48 b	93.38±15.91 b	93.88±8.03^{b}
C	97.50±5.00^{a} b	94.06±6.11^{b} c	87.36±12.07^{a} b	85.58±17.62 b	90.40±6.49 b	86.92±12.16 a	88.67±7.58^{a}
D	100.00±0.00 b	92.50±7.39 b	88.72±6.37^{ab}	93.49±11.18 b	92.49±3.53 b	97.24±2.48^{c}	95.08±1.68^{b}

*values followed by same superscript alphabets in a column are not significant different at p=0.05

TLD – Total Larval Survival rate
TID – Total Immature Survival rate
A = Gidan Kwano; B= Bosso; C= Maikunkele; D= Chanchaga

Illustration 2

Duration of Immature stages of (days) Culex pipiens pipiens in North central Nigeria during the rainy season of 2011

Larval Collection Site	Larval Instar Stages					Pupal Stage	TID
	L1	L2		L3			
	L4	TLD					
A	1.67±0.16[a]*	1.84±0.2[a]	1.93±0.34 a	2.30 ±0.45[a]	7.75±0.76[a] b	1.95±0.37 a	9.70±0.96[a] b
B	2.05±0.30[b]	2.03±0.39[a] b	2.14±0.42 a	2.21±0.40 a	8.44±0.72[b]	1.67±0.63 a	10.10±0.94 b
C	1.63±0.10[a]	1.46±0.20 a	1.60±0.45 a	2.40±0.42 a	7.04±1.47 a	1.58±0.62 a	8.67±2.03 a
D	1.20±0.16 [a]	2.51±0.53[b]	2.41±0.24 b	1.86±0.37 a	7.98±0.37[a] b	1.73±0.43 a	9.71±1.92[a] b

*values followed by same superscript alphabets in a column are not significant different at p=0.05

TLD – Total Larval Duration
TID – Total Immature Duration
A= Gidan Kwano; B= Bosso; C= Maikunkele; D= Chanchaga

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