Genetic Variations in Bionomics of *Culex quinquefasciatus* (Diptera: Culicidae) Mosquito Population in Minna, North Central Nigeria

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ABSTRACT: The need to have an improved knowledge on the bioecology of *Culex quinquefasciatus*, a prerequisite in the development of cost-effective control strategies, has informed the present preliminary investigation to put in better perspective variations that exist in the egg rafts of the species. Freshly laid egg rafts were collected and incubated at ambient temperature in well-labeled plastic trays. The results showed overall inconsistency in all indices monitored for the egg rafts. Generally, survivorship was high for the species. All immature stage and adult parameters measured varied significantly among the egg rafts and between/within sexes of the species. Therefore, this study suggests the presence of inherent variation in the bionomics of egg rafts of *C. quinquefasciatus*, probably influenced by the environment and hence underscores the need for additional studies to further elucidate the roles of genetics and environment in vectorial competence of the species, in order to develop robust sustainable mosquito vector control protocols.

KEYWORDS: intraspecific, environment, phenotype, genotype, postemergence

CITATION: Ukubuiwe et al. Genetic Variations in Bionomics of *Culex quinquefasciatus* (Diptera: Culicidae) Mosquito Population in Minna, North Central Nigeria. *International Journal of Insect Science* 2016:8 9–15 doi:10.4137/IJIS.S32516.

TYPE: Original Research

RECEIVED: August 4, 2015. **RESUBMITTED:** October 8, 2015. **ACCEPTED FOR PUBLICATION:** January 28, 2016.

ACADEMIC EDITOR: Emily Angiolini, Deputy Editor in Chief

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 1676 words, excluding any confidential comments to the academic editor.

FUNDING: This study was funded from an award obtained from the United States Agency for International Development (USAID)—University of Mississippi fellowship. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

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Introduction

Culex mosquitoes constitute a major threat to public health, being ranked with the genera of *Anopheles* and *Aedes* in the transmission of epidemiologically important diseases.[1–3] *Culex* mosquitoes, especially the species of *Culex quinquefasciatus*, have been incriminated in the transmission of diseases such as filariasis and elephantiasis in Africa in general and Nigeria in particular.[4–7] It is estimated that more than 1.3 billion people in 83 countries are at risk of infection.[8] Furthermore, the economic burden of these diseases is enormous, as countries such as Nigeria spend billions of public funds, which could have been channeled into other productive sectors of the economy, on fighting these diseases.[9] However, the epidemiology of the diseases in Nigeria is complicated,[6] owing to various factors that are predominantly related to the vector species involved, and these factors include ubiquitous distribution of the disease vector (which increases vector–human contacts),[10] catholic habitation (an inherent ability to survive in an assortment of habitats),[11] varying degree of anthropogenic activities (which provides varieties of suitable oviposition sites for adults and fitting habitats for the larval development),[12,13] clemency of the Afrotropical climate (which favors the rapid development of vectors and parasites),[14,15] and genetic variability.[10,16,17]

Among these factors, genetic variability (within and without offsprings/progeny) tends to be most important, as the genetic makeup of an individual species serves as a blue print for vectorial competence, although this may be highly modified by the environment. Genetic unevenness has been reported for different species of mosquitoes,[16] even for sibling species,[18] and from one locality to another.[19] Preliminary studies have shown that Minna is rich in mosquitoes species diversity[11,20,21] that have shown variations in survivorship and duration of development,[19,22–24] teneral reserve component,[17] and vectorial fitness attributes[17,19] of the species in Minna. In a bid to harmonize these variations, which will advance present knowledge about the species and fill existing gaps in our knowledge of variability within the species, baseline information needs to be generated and this has informed this study. In addition, gaps still exist in our knowledge of variability of egg rafts among individuals of a species/family for the vector species in Minna, North Central Minna.

Therefore, this study aims to bridge this gap of knowledge. It intends to elucidate inherent variations in the productivity of egg rafts (ie, proxy for reproductive viability), survivorship and duration of development of immature stages, and reproductive and vectorial fitness attributes that exist in the imagines from such egg rafts.

Materials and Methods

Study area. This study was carried out in Minna, the capital of Niger State, North Central Nigeria. Minna is located within longitude 6° 33′E and latitude 9° 27′N, covering a land area of 88 km^2 with an estimated human population of 1.2 million. The area is characterized by a tropical climate with mean annual temperature, relative humidity, and rainfall of 30.20°C, 61.%, and 1334.00 mm, respectively. The climate presents two distinct seasons in a year: a rainy season between May and October and a dry season between November and April. The vegetation in the area is typically grass-dominated savannah with scattered trees.[14]

Source and incubation of egg rafts. The city was combed thoroughly for egg rafts from conventional breeding habitats; recovered rafts were put in carefully labeled containers and transported to the entomological unit of the Department of Biological Sciences. The egg rafts were screened and identified for those belonging to *C. quinquefasciatus* using the techniques described by Weber and Weber[25] and incubated according to the techniques of De Meillon and Thomas,[26] with slight modifications. Briefly, 10 egg rafts were simultaneously placed in the well-labeled transparent plastic hatching trays (A–J), filled with 250 mL of borehole water as culture medium and incubated at ambient room conditions of 28.00 ± 1.00°C, 70.20 ± 2.82% RH, and 12L:12D photoperiod. After hatching, the species were confirmed using the morphological keys suggested by Hopkins.[27] The number of larvae that hatch out of each egg raft was used as an estimate of its productivity/hatchability. Those rafts that did not hatch after 72–96 hours were considered nonviable and were appropriately dispensed.

Larvae culture. The larvae were reared following standard techniques in plastic bowls (1250 mL capacity) at the rate of 250 larvae/bowl in 1000 mL of borehole water. The larvae were fed with fish feed (TetraMin®), at the rate of 0.32 mg/larva every other day, sprinkled on the water surface. On every alternate day, the water from the culture bowls was changed till pupation.[19,28]

Pupae culture. The pupae were separated daily and placed in plastic bowls (5 cm in height and 20 cm in diameter) half filled with borehole water. The plastic bowls with pupae were labeled and placed in adult-holding cages for emergence, and mortality was noted; pupae that were unable to emerge or adults that were unable to break free from the pupal case were considered dead.[19,29]

Duration and survivorship of immature stages. The duration of the immature stages and stage-specific survivorship were computed according to the life table analysis suggested by Olayemi and Ande.[14] 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.[19]

Sex ratio and adult longevity. The sex of each emergent mosquito was noted, and all adult mosquitoes were subsequently monitored for daily survival rates and life span. During this period, the mosquitoes were fed with only sugar solution (10%) soaked in cotton wool.[30]

Adult fitness attributes (adult wing length and fluctuating asymmetry). The adult fitness attribute, which is basically a measure of how well formed a mosquito is, to fly, locate mate, locate host, bite, and transmit pathogen, was estimated from data on body size and symmetry of the wings. The body size of adult mosquitoes is reflected by the wing size, which was determined as described by Gafur[16] and Ukubuiwe et al.[17] The wings of emergent mosquitoes were carefully detached with the aid of entomological pins. The left and right wings were preserved in separate envelopes for further analysis. Wing length was measured according to the techniques of Gafur,[16] and fluctuating asymmetry (FA) was determined as the difference between the left and right wings.

Data analyses. Differences in the mean values of duration and survival rates of immature life stage, as well as adult wing lengths, among the egg rafts were compared for statistical significance using analysis of variance at $P = 0.05$. All data obtained from experimental replicates and repeats were processed as mean ± SD and subsequently pooled for statistical analysis. The mean values were separated using the Duncan's multiple range test.

Results

Egg raft productivity and duration of immature life stages. Egg raft fertility and developmental rates among the families of *C. quinquefasciatus* population in Minna are presented in Table 1. Egg raft fertility in the mosquitoes was 70.%, and 173.14 ± 47.72 larvae hatched per raft. The number of larvae hatched per raft varied significantly ($P < 0.05$) and ranged from 104 in raft number 8 (RN8) to 224 in RN5. Apart from second larval instar (L2), significant ($P < 0.05$) family variation in the duration of immature stages was observed among the egg rafts. Mean values of the results showed that the duration of development of the specific larval instars of the mosquito population increased steadily from L1 (1.66 ± 0.27 days) to L3 (2.33 ± 0.42 days) but drastically in L4 (4.09 ± 1.43 days): L1 (ranging from 1.39 ± 0.25 days in RN3 to 1.94 ± 0.22 days in RN6), L2 (ranging from 1.82 ± 0.31 days in RN10 to 2.20 ± 0.37 days in RN8), L3 (ranging from 1.81 ± 0.09 days in RN8 to 3.23 ± 0.43 days in RN10), and L4 (ranging from 1.79 ± 0.54 days in RN8 to 5.78 ± 1.01 days in RN10). Furthermore, total larval duration in the population was 10.09 ± 1.43 days and equally ranged from 6.83 ± 0.77 days in RN8 and 12.78 ± 1.16 days in RN10, representing egg rafts/families with the fastest and slowest developing larvae, respectively.

Like larval development, the duration of pupal stage (PSD) and total immature duration (TID) differed significantly among the mosquito families, averaging 0.97 ± 0.24 days and 11.07 ± 1.48 days, respectively. Significant ($P < 0.05$) familial variation in PSD witnessed RN1 and RN6 producing pupae that spent the shortest (0.51 ± 0.59 days) and longest

Table 1. Hatchability of egg rafts and mean ± SD of duration (days) of immature stages (progeny) of egg rafts of *C. quinquefasciatus* in Minna, Nigeria.

EGG RAFT	NUMBER OF HATCHED LARVAE	LARVAL STAGES DURATION				TOTAL LARVAL DURATION	PUPAL STAGE DURATION	TOTAL IMMATURE DURATION
		L1	L2	L3	L4			
RN1	$208^{e,*}$	$1.76 \pm 0.56^{a,b,*}$	2.17 ± 0.19^{a}	2.35 ± 0.46^{b}	$2.82 \pm 3.34^{a,b}$	9.10 ± 2.69^{b}	0.51 ± 0.59^{a}	$9.61 \pm 3.24^{a,b}$
RN2	0^{a}	–	–	–	–	–	–	–
RN3	166^{d}	1.39 ± 0.25^{a}	2.09 ± 0.32^{a}	2.01 ± 0.67^{a}	$5.32 \pm 1.44^{b,c}$	$10.82 \pm 1.75^{b,c}$	1.02 ± 0.18^{b}	$11.84 \pm 1.74^{b,c}$
RN4	220^{f}	$1.85 \pm 0.19^{a,b}$	2.01 ± 0.14^{a}	$2.17 \pm 0.31^{a,b}$	4.80 ± 1.56^{b}	$10.84 \pm 1.36^{b,c}$	1.01 ± 0.15^{b}	$11.85 \pm 1.46^{b,c}$
RN5	224^{f}	$1.67 \pm 0.33^{a,b}$	1.87 ± 0.34^{a}	1.93 ± 0.55^{a}	$3.79 \pm 1.11^{a,b}$	9.27 ± 0.99^{b}	$1.13 \pm 0.29^{a,b}$	10.39 ± 0.77^{b}
RN6	170^{d}	1.94 ± 0.22^{b}	1.94 ± 0.11^{a}	$2.80 \pm 0.43^{b,c}$	4.34 ± 0.99^{b}	$11.01 \pm 1.26^{b,c}$	1.23 ± 0.29^{c}	$12.24 \pm 1.27^{b,c}$
RN7	0^{a}	–	–	–	–	–	–	–
RN8	104^{b}	1.41 ± 0.23^{a}	2.20 ± 0.37^{a}	1.81 ± 0.09^{a}	1.79 ± 0.54^{a}	6.83 ± 0.77^{a}	$1.17 \pm 0.05b^{a,b}$	8.00 ± 0.67^{a}
RN9	0^{a}	–	–	–	–	–	–	–
RN10	120^{c}	$1.57 \pm 0.11^{a,b}$	1.82 ± 0.31^{a}	3.23 ± 0.43^{c}	5.78 ± 1.01^{c}	12.78 ± 1.16^{c}	1.05 ± 0.11^{b}	13.83 ± 1.18^{c}
Aggregate	173.14 ± 47.72	1.66 ± 0.27	2.01 ± 0.25	2.33 ± 0.42	4.09 ± 1.43	10.09 ± 1.43	0.97 ± 0.24	11.07 ± 1.48

Notes: RN1–10 = raft number 1–10; and L1–L4 = first to fourth larval instar. *Values followed by same superscript alphabets in a column are not significantly different at $P = 0.05$.

(1.23 ± 0.29) time, respectively. These differences translated into various familial TIDs, with larvae in RN8 having the shortest TID (8.00 ± 0.67 days), were closely followed by those in RN1 (9.61 ± 3.24 days). RN10 produced larvae that spent the longest time (13.83 ± 1.18 days) in immature development.

Survivorship of immature life stage. Survivorship of the immature life stage is highlighted in Table 2. Mean aggregate survivorship of the immature mosquitoes was 89.91 ± 5.08% and was higher in the pupal stage (95.88 ± 3.16%) than that in the larval stage (88.42 ± 6.07%). Unlike the duration of development, survivorship of larval instars decreased steadily from L1 (98.29 ± 1.64%) to L3 (89.23 ± 8.78%) and, then, drastically in L4 (72.52 ± 17.02%). Differences within families of the species were significant ($P < 0.05$) and worthy of note during the larval instar development than at the pupal stage; however, the differences were generally low for immature survivorship. Analyses of data between the families revealed the greatest survivorship in the first instar larvae, L1 (with values >98% in RN1, RN6, and RN8 and 100.% in RN3, RN4, and RN5), which was closely followed by the pupal life stages (survivorship: <90.% in RN8 and RN10 and >95% in RN1, RN3, RN4, RN5, and RN6). Conversely, L4 stages were the least survived (<50% in RN1 and <50% in RN3, RN4, RN5, RN6, RN8, and RN10). Apart from larvae from RN1, there was generally no significant difference ($P > 0.05$) in the average larval survival rates and average immature survival rates within the families, with values >80% and 85%, respectively (Table 2).

Adult emergence rate and survivorship. Table 3 shows the number of emergent imagines from the egg rafts, their daily survivorship, average postemergence longevity (APL) of adult mosquitoes, and their average life expectancy. Analyses

Table 2. Mean ± SD of stage-specific survivorship (%) of immature stages (progeny) of egg rafts of *C. quinquefasciatus* in Minna, Nigeria.

EGG RAFT	LARVAL STAGES				AVERAGE LARVAL SURVIVAL RATE	PUPAL STAGE SURVIVAL RATE	AVERAGE IMMATURE SURVIVAL RATE
	L1	L2	L3	L4			
RN1	$98.00 \pm 4.00^{a,b,*}$	75.22 ± 17.15^{a}	65.65 ± 40.47^{a}	41.13 ± 32.84^{a}	69.99 ± 18.97^{a}	100.00 ± 0.00^{b}	75.99 ± 15.18^{a}
RN3	100.00 ± 0.00^{b}	99.00 ± 2.00^{b}	97.92 ± 2.41^{b}	$65.18 \pm 29.00^{a,b}$	90.52 ± 7.45^{b}	100.00 ± 0.00^{b}	92.42 ± 5.96^{b}
RN4	100.00 ± 0.00^{b}	96.00 ± 5.66^{b}	$80.29 \pm 3.17^{a,b}$	$62.74 \pm 11.49^{a,b}$	84.76 ± 1.79^{b}	100.00 ± 0.00^{b}	87.81 ± 1.44^{b}
RN5	100.00 ± 0.00^{b}	99.00 ± 2.00^{b}	99.00 ± 2.00^{b}	93.83 ± 4.22^{b}	97.96 ± 1.47^{b}	98.91 ± 2.18^{b}	98.15 ± 1.26^{b}
RN6	$98.00 \pm 0.00^{a,b}$	99.00 ± 2.00^{b}	96.83 ± 4.14^{b}	88.39 ± 18.09^{b}	95.55 ± 6.30^{b}	100.00 ± 0.00^{b}	96.44 ± 5.04^{b}
RN8	$98.00 \pm 2.31^{a,b}$	92.83 ± 8.49^{b}	95.12 ± 7.07^{b}	$62.53 \pm 19.08^{a,b}$	87.12 ± 3.69^{b}	90.89 ± 7.07^{b}	87.87 ± 3.29^{b}
RN10	94.00 ± 5.16^{a}	94.43 ± 5.75^{b}	$89.83 \pm 2.18^{a,b}$	93.86 ± 4.44^{b}	93.03 ± 2.80^{b}	81.39 ± 12.89^{a}	90.70 ± 3.37^{b}
Aggregate	98.29 ± 1.64	93.64 ± 6.15	89.23 ± 8.78	72.52 ± 17.02	88.42 ± 6.07	95.88 ± 3.16	89.91 ± 5.08

Notes: RN1–10 = raft number 1–10; L1–L4 = first to fourth larval instar. *Values followed by same superscript alphabets in a column are not significantly different at $P = 0.05$.

Table 3. Adult mosquito emergence rate and longevity of *C. quinquefasciatus* in Minna, Nigeria.

EGG RAFT	NUMBER OF EMERGENT IMAGOS* (PER EGG RAFT)			DAILY SURVIVORSHIP OF IMAGINES (%)*			AVERAGE POST-EMERGENCE LONGEVITY (APL) (DAYS)		
	MALE	FEMALE	TOTAL	MALE	FEMALE	MEAN	MALE	FEMALE	MEAN
RN1	$13^{a,**}_{a}$	$10^{a}_{a,***}$	23	$41.71 \pm 35.42^{a}_{a}$	$45.33 \pm 37.22^{a}_{b}$	43.52 ± 36.32	$6.38 \pm 8.40^{b}_{a}$	$6.10 \pm 7.38^{a}_{a}$	6.24 ± 7.89
RN3	25^{c}_{a}	38^{c}_{b}	63	$43.25 \pm 35.73^{a}_{a}$	$64.25 \pm 34.27^{c}_{b}$	53.75 ± 35.00	$9.20 \pm 4.03^{d}_{b}$	$7.71 \pm 4.78^{c}_{a}$	8.46 ± 4.41
RN4	20^{b}_{a}	28^{b}_{b}	48	$53.57 \pm 36.80^{b}_{a}$	$54.04 \pm 36.31^{b}_{a}$	53.81 ± 36.56	$4.55 \pm 2.90^{a}_{a}$	$7.50 \pm 3.78^{c}_{b}$	6.03 ± 3.34
RN5	35^{d}_{a}	56^{e}_{b}	91	$71.17 \pm 31.64^{d}_{a}$	$70.62 \pm 31.58^{c}_{a}$	70.89 ± 31.62	$11.25 \pm 7.17^{e}_{a}$	$11.13 \pm 9.47^{e}_{a}$	11.19 ± 8.32
RN6	37^{d}_{a}	47^{d}_{b}	84	$60.24 \pm 37.47^{c}_{a}$	$65.80 \pm 33.13^{c}_{b}$	63.02 ± 35.24	$8.76 \pm 3.89^{d}_{a}$	$10.09 \pm 4.83^{d}_{b}$	9.42 ± 4.36
RN8	21^{b}_{a}	27^{b}_{a}	48	$51.38 \pm 34.17^{b}_{b}$	$44.62 \pm 37.21^{a}_{a}$	48.00 ± 23.59	$6.97 \pm 3.39^{c}_{a}$	$6.56 \pm 2.74^{b}_{a}$	6.77 ± 3.07
RN10	26^{c}_{a}	35^{c}_{b}	61	$50.49 \pm 35.97^{b}_{a}$	$59.65 \pm 33.98^{b,c}_{b}$	55.08 ± 34.98	$6.89 \pm 2.45^{c}_{a}$	$7.49 \pm 3.03^{c}_{b}$	7.19 ± 4.96
Aggregate	25.29 ± 8.46	34.43 ± 14.89	59.72 ± 23.55	$53.12 \pm 35.36_{a}$	$57.76 \pm 34.81_{b}$	55.44 ± 33.32	7.72 ± 3.41	8.08 ± 3.83	7.89 ± 5.19

Notes: RN1–10 = raft number 1–10. *Total number of adults monitored for each egg raft = 100. **Values followed by same superscript alphabets in a column are not significantly different at $P = 0.05$. ***Values followed by same subscript alphabets in a row for each male and female are not significantly different at $P = 0.05$.

showed a significant variation ($P < 0.05$) in these parameters for egg rafts/families and between/within sexes of egg rafts. Apart from RN1 and RN8, other rafts recorded significant difference ($P < 0.05$) in the number of emergent male and female adults, ranging from 13 in RN1 to 37 in RN6 and 10 in RN1 to 56 in RN5, respectively. Adult mosquito emergence rates ranged from 23 to 91 imagines per egg raft, with an average of 59.72 ± 23.35 for the mosquito species (Table 3). Sex-wise distribution of the imagines varied significantly among the families (ie, egg rafts). Significantly higher number of female mosquitoes (mean = 34.43 ± 14.89 mosquitoes/egg raft) than the male mosquitoes (mean = 25.29 ± 8.46 mosquitoes/egg raft) were recorded.

The influence of family source on daily survivorship of the adult mosquitoes was also significant ($P < 0.05$) and ranged from $41.71 \pm 35.42\%$ (in RN1) to $71.17 \pm 31.64\%$ (in RN5) and $44.62 \pm 37.21\%$ (in RN8) to $70.62 \pm 31.58\%$ (in RN5) for the male and female mosquitoes, respectively. On the whole, daily survivorship of the female mosquitoes ($57.76 \pm 34.81\%$) was significantly higher than that of the male mosquitoes ($53.12 \pm 35.36\%$), thus revealing the variation among families; apart from RN5, other rafts had significant difference ($P < 0.05$) in the survivorship of male and female mosquitoes. Generally, RN5 imagines were the most daily survived for the sexes, and RN1 adults being the least survived (Table 3).

Life expectancy (ie, longevity) of the male and female mosquitoes followed the same pattern as daily survival rate, with longevity of 7.72 ± 3.41 and 8.08 ± 3.83 days, respectively. Analyses of APL revealed variation between and among sexes of the egg rafts, with RN5 producing adults with the longest APL for males (11.25 ± 7.17 days) and females (11.13 ± 9.47 days). These variations translated into mean APL for the species as 7.89 ± 5.19 days, ranging from 6.24 ± 7.89 days in RN1 to 11.19 ± 8.32 days in RN5 (Table 3).

Wing length and FA. Wing length (proxy for adult body size) was longer in the female mosquitoes (3.15 ± 0.18 mm) than male mosquitoes (2.97 ± 0.12 mm) (Table 4). However, the left and right wing lengths in both sexes varied within narrow limits. Noticeably, family source had significant ($P < 0.05$) influence on wing lengths of the mosquitoes, as the biggest male mosquitoes were from families/egg rafts RN1 and RN10 (mean, 3.20 ± 0.03 mm and 3.23 ± 0.10 mm, respectively), while RN8 produced the smallest male mosquitoes (mean, 2.61 ± 0.14 mm). In addition, RN1 and RN10 produced the biggest female mosquitoes (mean, 3.27 ± 0.09 mm and 3.24 ± 0.18 mm, respectively), but unlike for the male mosquitoes, RN5 produced the smallest female mosquitoes (mean, 2.92 ± 0.15 mm). This result is further buttressed by the analyses of aggregate adult wing lengths (male and female), which revealed that RN1 and RN10 produced the largest mosquitoes, while RN5 produced the smallest mosquitoes. FA of the wings was very low (aggregate population mean = 0.02 mm) and similar for both sexes.

Table 4. Wing length and FA of *C. quinquefasciatus* in Minna, Nigeria.

EGG RAFT	WING LENGTH (mm)							AGGREGATE (MALE AND FEMALE)			
	MALE				FEMALE						
	LEFT	RIGHT	MEAN	FA**	LEFT	RIGHT	MEAN	FA	LEFT	RIGHT	MEAN	FA
RN1	$3.23 \pm 0.02^{d,*}$	3.17 ± 0.03^{d}	3.20 ± 0.03^{d}	0.01	3.25 ± 0.04^{b}	3.28 ± 0.13^{c}	3.27 ± 0.09^{d}	0.02	3.24 ± 0.23	3.23 ± 0.08	3.24 ± 0.31	0.02
RN3	3.12 ± 0.01^{c}	3.09 ± 0.03^{c}	3.11 ± 0.02^{c}	0.02	3.23 ± 0.12^{b}	3.17 ± 0.18^{b}	3.20 ± 0.15^{c}	0.01	3.18 ± 0.07	3.13 ± 0.11	3.16 ± 0.18	0.02
RN4	2.89 ± 0.21^{b}	2.86 ± 0.23^{b}	2.88 ± 0.22^{b}	0.00	$2.98 \pm 0.19^{a,b}$	$2.97 \pm 0.23^{a,b}$	2.98 ± 0.21^{b}	0.01	2.94 ± 0.20	2.92 ± 0.23	2.93 ± 0.22	0.005
RN5	$2.78 \pm 0.17^{a,b}$	2.83 ± 0.20^{b}	$2.81 \pm 0.19^{a,b}$	0.01	2.93 ± 0.10^{a}	2.90 ± 0.19^{a}	2.92 ± 0.15^{a}	0.02	2.86 ± 0.13	2.87 ± 0.20	2.87 ± 0.16	0.02
RN6	2.92 ± 0.16^{b}	2.97 ± 0.12^{c}	2.95 ± 0.14^{c}	0.00	3.23 ± 0.20^{b}	3.24 ± 0.31^{b}	3.24 ± 0.26^{c}	0.02	3.08 ± 0.18	3.11 ± 0.22	3.09 ± 0.20	0.01
RN8	2.62 ± 0.09^{a}	2.60 ± 0.08^{a}	2.61 ± 0.14^{a}	0.00	$3.17 \pm 0.23^{a,b}$	3.19 ± 0.27^{c}	3.18 ± 0.25^{c}	0.01	2.90 ± 0.17	2.89 ± 0.18	2.90 ± 0.18	0.01
RN10	3.27 ± 0.10^{d}	3.19 ± 0.09^{d}	3.23 ± 0.10^{d}	0.00	3.23 ± 0.13^{b}	3.24 ± 0.22^{c}	3.24 ± 0.18^{d}	0.02	3.25 ± 0.12	3.22 ± 0.16	3.24 ± 0.14	0.01
Aggregate	2.98 ± 0.11	2.96 ± 0.11	2.97 ± 0.12	0.02	3.15 ± 0.14	3.14 ± 0.22	3.15 ± 0.18	0.01	3.07 ± 0.13	3.05 ± 0.17	3.06 ± 0.15	0.02

Notes: RN1–10 = raft number 1–10; and FA = fluctuating asymmetry. *Values followed by same superscript alphabets in a column are not significantly different at $P = 0.05$. **Difference between left and right wing ratios.

Discussion

Knowledge of genetic diversity present within families of a species is important in understanding the evolutionary adaptability of the organism to function well in its ecological niche. The life cycle of mosquitoes starts from the laid egg (singly or in rafts), and life attributes of the progeny depend largely on the quality of the eggs. In the present study, which is a preliminary investigation, the egg rafts were not all productive, as 30% of the randomly collected eggs were not viable; this confirms earlier assumptions that not all egg rafts are viable. However, egg raft fertility was relatively high (70%) for the species (*C. quinquefasciatus* population) in the area, indicating an ecologically well-adapted and genetically fit mosquito population. These are bases for rapid development of high *C. quinquefasciatus* population density in Minna. This result explains the preponderance of *Culex* species especially *C. quinquefasciatus* in previous mosquito collections in the area.[11,15,20] High population density of *C. quinquefasciatus* in Minna poses serious threat to public health especially with the transmission of filariasis and elephantiasis.[31,32]

Duration and survivorship of immature stages of the mosquito species averaged about 11.07 ± 1.48 days and 90%, respectively. These results are similar to those of Olayemi et al,[22] who reported values of 11.57 ± 0.00 days and 90.38 ± 6.52%, respectively. However, their results contradicted those reported by Ukubuiwe et al,[19] who reported a duration of development of 8.67 ± 2.03 to 10.10 ± 0.94 days and survivorship range of 88.87 ± 7.58% to 95.08 ± 1.68% for species collected from different areas of the city. In addition, they differed from those reported by Olayemi et al,[23] who reported survivorship of 95.40 ± 2.87% and duration of immature development of 9.97 ± 0.74 days for the same species. These similarities/dissimilarities may be due to genetic and/or environmental concurrence/differences between the populations of mosquito species, as they were raised in the same laboratory conditions.

In the present study, familial variation in the immature duration showed that mosquitoes from RN8 are fast developers, while those from RN10 are very slow developers (eliminating the influence of habitat source by rearing in the same breeding conditions), which is of great importance epidemiologically and entomologically, as it can affect the prediction of population explosion from existing data. Thus, it points to a differential endowment of the species genetically; this could have been the reason for the conflicting results from previous studies on the species.

Increased duration of instar stage with larval age as reported in this study and supported by earlier studies[19,22–24] may be occasioned by the increasing need to accumulate teneral reserve for egg development in female mosquitoes during the adult stage. In addition, Briegel et al[33] and Briegel[34] reported the climax teneral reserve accumulation in mosquitoes during the L4 instar stage, which in the present study

varied significantly between families/egg rafts and could signify differential accumulation tendencies.

While the duration of larval instar stage increased from L1 to L4, the reverse was the case with survival rates. The decreasing survivorship of the larval instars (of all the families/egg rafts) with age (ie, L1–L4) seems to disfavor the ecological adaptability of *C. quinquefasciatus* in Minna, as indicated by the high fertility of the egg rafts and reported dominance in the area. However, the explanation may be found in the differences between laboratory-reared conditions of the larvae as against the field environmental conditions to which the species has probably adapted, especially as the egg rafts used in this experiment came from the wild and not from a laboratory-adapted mosquito colony.

Significantly, higher densities of and better survivorship of female mosquitoes per egg raft that varied among egg rafts/families were not unexpected, as nature, through evolutionary selection, tends to invest more in the female sex of many species, as they are regarded as better assets. For example, female mosquito needs to produce eggs (a costly physiological and metabolic process), accumulate more teneral reserve during the larval stage,[35] and take blood meals (a highly proteinous food source), which stand them in good stead for better adult life performance than the males.[34,36,37]

Wing length of mosquitoes gives an estimate of body weight[38] and body size[39] and affects longevity, fecundity, and blood meal volume, which may influence the fitness of the vector for disease/parasite transmission. In addition, FA measures deviation from perfect bilateral symmetry caused by environmental and/or genetic stress experienced during ontogeny and usually is an interaction between the environment and the genotype or wholly genetic expression.[40,41] In the present study, differential wing lengths were noted across the families and the earlier productive and perceived genetically efficient egg raft (RN5; with the greatest immature survivorship, highest adult emergence, and longest surviving mosquitoes) produced the smallest mosquitoes with greater FA, with the female mosquitoes having longer wing lengths (ie, adult body size) than the male mosquitoes. This is supported by earlier studies by Agnew et al[42] and Kaufmann and Briegel,[37] who reported greater value of wing length for female species for *Stegomyia aegypti* (L.), *Stegomyia albopicta*, *Ochlerotatus. triseriatus* (Say), *C. quinquefasciatus* Say, and *Culex salinarius* Coquillet.

Although familial variation exists, the FA of the wings of *C. quinquefasciatus* population in Minna was generally very low (and significantly differed between both sexes), which may suggest the absence of significant environmental stress and/or high ecological adaptability of the wild mosquito species in the area. This again explains the preponderance of *C. quinquefasciatus* species in Minna, with its attendant epidemiological consequences for disease transmission. These deductions are supported by earlier findings of Imasheva et al[43] and Mpho et al[41] on FA as a good indicator of the occurrence of environmental stress on mosquito populations in an area.

The influence of family variations on the reproductive and adult fitness of *C. quinquefasciatus* studied was significant. This finding indicates the need for commensurate studies on the genetics of mosquitoes and influence of environmental factors to ascertain their roles in the vector fitness and disease transmission ability of the vector. The ecological implication of the existing significant diversity in *C. quinquefasciatus* population in Minna is that the population of the mosquito is still evolving and is therefore expected to get better adapted to the environmental conditions in the area with time. This must not be allowed to happen in order to forestall the potential intolerably high *Culex*-borne disease burdens associable with such scenarios.

Conclusion

This preliminary study shows that *C. quinquefasciatus* mosquito population in Minna, Nigeria, is diversified, vigorous, and ecologically well adapted to the prevailing environmental factors in the area. The population seems not to be under significant environmental stress, and evolutionary forces have significantly equipped and selected female individuals over the males. However, further studies are advocated to substantiate these aspects. Family divergence in reproductive and vectorial fitness traits of the species are high, indicating that the mosquito is likely to become a much better vector with time, thus constituting serious public health threat in the area. Therefore, the findings of this study underscore the need for further studies on the role of genetics and/or environment on vectorial importance of mosquito species, and the results of such studies should help in fine-tuning the development of robust sustainable mosquito vector control protocols.

Acknowledgment

We wish to acknowledge the management and staff of the Department of Biological Sciences, Federal University of Technology, Minna, for the unrestricted use of the equipment and space in the laboratory.

Author Contributions

Conceived and designed the experiment: ACU and IKO. Analyzed the data: ACU. Wrote the first draft of the manuscript: ACU. Contributed to the writing of the manuscript: IKO and AIJ. Agreed with manuscript results and conclusion: ACU, IKO, and AIJ. Jointly developed the structure and arguments for the paper: ACU, IKO, and AIJ. Made critical revisions and approved the final version: all authors. All the authors reviewed and approved the final manuscript.

REFERENCES

1. Lane RP, Crosskey RW. *Medical Insects and Arachnids*. London: Chapman and Hall; 1993.
2. Sachs J, Malaney P. The economic and social burden of malaria. *Nature*. 2002;415:680–685.

3. World Health Organization. *World Malaria Report 2008*. Geneva: World Health Organization; 2008.
4. Anosike JC, Nwoke BE, Ajayi EG, et al. Lymphatic filariasis among the Ezza people of Ebonyi State, Eastern Nigeria. *Ann Agric Environ Med*. 2005; 12:181–186.
5. Ibanga UN, Braide EI, Okpara KN, Atting IA, Adie HA. Current status of bancroftian filariasis in rural communities of the lower cross river basin, Nigeria: parasitological and clinical. *J Public Health*. 2008;16(6):383–388.
6. Obi RK, Nwanebu FC, Ndubuisi-Nnaji UU, et al. Endemicity of lymphatic filariasis in three local government areas of Imo State, Nigeria. *Niger J Parasitol*. 2010;31(1):26–30.
7. Omudu EA, Okafor FC. A comparative study of chronic lymphatic filariasis-related knowledge, attitudes and perception among three ethnic groups in Benue State, Nigeria. *Niger J Parasitol*. 2010;31(1):14–20.
8. World Health Organisation. *Community Participation and Tropical Disease Control in Resource-Poor Settings*. Geneva: World Health Organization; 2004. TDR/STR/SEB/ST/04.1.
9. Carter Centre. Lymphatic Filariasis Elimination Program. 2008. Available at: http://www.cartercenter.org/health/lf/index.html. Accessed August, 28 2011.
10. Aaron CB. Changing patterns of West Nile virus transmission: altered vector competence and host susceptibility. *Vet Res*. 2009;40:43.
11. Olayemi IK, Ukubuiwe AC, Oyibo-Usman KA. Mosquito species occurrence and diversity in conventional larval breeding sites in Minna metropolis, Nigeria. *Int J Innov Sci Res*. 2014;9(1):86–93.
12. Muturi EJ, Burgess P, Navak RJ. Malaria vector management: where have we come from and where are we headed? *Am J Trop Med Hyg*. 2008;78:536–537.
13. Olayemi IK. Influence of land-use on the fitness of *Anopheles gambiae*, the principal vector of malaria in Nigeria. *Online J Health Allied Sci*. 2008;7(4):3.
14. Olayemi IK, Ande AT. Life table analysis of *Anopheles gambiae* (Diptera: Culicidae) in relation to malaria transmission. *J Vector Borne Dis*. 2009;46:295–298.
15. Olayemi IK, Idris B, Ejima IAA, Adeniyi K, Ukubuiwe AC, Isah B. The climate of North-central Nigeria and potential influence on mosquito (Diptera:Culicidae) vectorial capacity, for disease transmission. *Global J Multidiscip Appl Sci*. 2014;2(2):26–31.
16. Gafur A. Discrimination of female *Aedes aegypti* (Diptera: Culicidae) from Banjarmasin and Yogyakarta based on wing measurements. *Bioscientiae*. 2004; 1(2):41–53.
17. Ukubuiwe AC, Olayemi IK, Omalu ICJ, Jibrin AI, Oyibo-Usman KA. Molecular bases of reproductive and vectorial fitness of *Culex pipiens pipiens* (Diptera: Culicidae) mosquito populations, for the transmission of filariasis in North Central Nigeria. *J Med Sci*. 2013;13(3):201–201.
18. Bryne K, Nichols RA. *Culex pipiens* in London Underground tunnels: Differentiation between surface and subterranean populations. *Heredity*. 1999;82:7–15.
19. Ukubuiwe AC, Olayemi IK, Omalu ICJ, Odeyemi MO, Jibrin AI, Oyibo-Usman KA. Comparative assessment of immature survivorship and developmental duration of *Culex pipiens pipiens* (Diptera: Culicidae) populations in north central Nigeria. *Biomed Cent Epidemiol*. 2012;3(10):WMC003753.
20. Olayemi IK, Olupinla T, Ukubuiwe AC, Odeyemi MO, Salihu IM. Distribution and oviposition dynamics of mosquito (Diptera: Culicidae) in relation to ovitrap substratal material in Minna, Nigeria. *Malaya J Biosci*. 2014;1(2):117–125.
21. Olayemi IK, Abdullahi-Sani H, Ukubuiwe AC, Adeniyi KA, Jibrin AI. Influence of ecological setting on occurrence of artificial container-breeding vector mosquito species (Diptera: Culicidae) and oviposition attraction to mineral salts in larval habitats, in Minna, North-central Nigeria. *Int J Innov Sci Res*. 2014;9(1):94–99.
22. Olayemi IK, Maduegbuna EN, Ukubuiwe AC, Chukwuemeka VI. Laboratory studies on developmental responses of the filarial vector mosquito, *Culex pipiens pipiens* (Diptera: Culicidae), to urea fertilizer. *J Med Sci*. 2012;12:175–181.
23. Olayemi IK, Yakubu H, Ukubuiwe AC. Larvicidal and insect growth regulatory (IGR) activities of leaf-extract of *Carica papaya* against the filariasis vector mosquito, *Culex pipiens pipiens* (Diptera: Culicidae). *Acta Malays*. 2013;2(3): 100–106.
24. Olayemi IK, Akpan B, Ejima IAA, Ukubuiwe AC, Olorunfemi OJ. Influence of rice-farming herbicide (2, 4-dichlorophenoxyl acetic acid) on the development of *Culex pipiens pipiens* (Diptera: Culicidae), a major swamp-breeding mosquito vector of filariasis. *Adv Agric Biol*. 2014;1(3):131–134.
25. Weber RM, Weber RG. The egg raft seam as an indicator of species in *Culex pipiens* and *Culex restuans*. *Mosq Syst*. 1985;17(4):363–370.
26. De Meillon B, Thomas V. *Culex pipiens* fatigans Wied. In: Smith CN, ed. *Insect Colonization and Mass Production*. New York: Academic Press; 1966:101–114.
27. Hopkins GHE. *Mosquitoes of Ethiopian Region. Larval Bionomics of Mosquitoes and Taxonomy of Culicinae Larvae*. 2nd ed. London: Adlard and Sons Ltd; 1952:1–355.
28. Das S, Garyer L, Dimopoulos G. Protocol for mosquito rearing (*An. gambiae*). *J Vis Exp*. 2007;5:221–225.
29. Gerberg EJ. Manual for mosquito rearing and experimental techniques. *Am Mosq Control Assoc Bull*. 1970;5:109.
30. Olayemi IK, Ande AT. Species composition and larval habitats of mosquitoes (Diptera: Culicidae) in Ilorin, Nigeria. *Zoologist*. 2008;6:7–15.
31. Kumar R. *Insect Pest Control: With Special Reference to African Agriculture*. London: Arnold; 1984:1–275.
32. Pfadt RE. *Fundamental of Applied Entomology*. 4th ed. New York: Macmillan Publishing Company; 1985:1.
33. Briegel H, Hefti M, DiMarco E. Lipid metabolism during sequential gonotrophic cycles in large and small female *Aedes aegypti*. *J Insect Physiol*. 2002;48:547–554.
34. Briegel H. Physiological bases of mosquito ecology. *J Vector Ecol*. 2003;28:1–11.
35. Briegel H. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *J Insect Physiol*. 1990;36:165–172.
36. Briegel H. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J Med Entomol*. 1990;27:839–850.
37. Kaufmann C, Briegel H. Flight performance of the malaria vectors *Anopheles gambiae* and *Anopheles atroparvus*. *J Vector Ecol*. 2004;29:140–153.
38. Alexandre C. Correlation between wing measurements and dry body weight in male and female *Ochlerotatus* (*Ochlerotatus*) *caspius* (Diptera: Culicidae). *Eur Mosq Bull*. 2007;24:4–8.
39. Siegel J, Novak R, Ruesink W. Relationship between wing length and dry weight of mosquitoes. *Journal of the American Mosquito Control Association*. 1994; 10(2):186–196.
40. Anderson WW. Genetic divergence in body size among experimental populations of *Drosophila pseudoobscura* kept at different temperature. *Evolution*. 1973;27:278–284.
41. Mpho M, Callaghan A, Holloway GJ. Effects of temperature and genetic stress on life history and fluctuating wing asymmetry in *Culex pipiens* mosquito. *Eur J Entomol*. 2002;99:405–412.
42. Agnew P, Hide M, Sidobre C, Michalakis Y. A minimalist approach to the effects of density-dependent competition on insect life history traits. *Ecol Entomol*. 2002;27:396–402.
43. Imasheva AG, Loeschcke V, Zhivotovsky LA, Lazebny OE. Effects of extreme temperature on phenotypic variation and developmental stability in *Drosophila melanogaster* and *Drosophila buzzatii*. *Biol J Linn Soc*. 1997;61:117–126.