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Molecular Bases of Reproductive and Vectorial Fitness of *Culex pipiens pipiens* (Diptera: Culicidae) Mosquito Populations, for the Transmission of Filariasis in North Central Nigeria

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The spatial heterogeneity of larval teneral reserves and their influence on wing attribute indices of adult vectorial fitness of *Culex pipiens pipiens* mosquitoes in North central Nigeria were elucidated in this study. Late 4th instar larvae of the mosquito were collected from four different populations in the area and analysed for nutritional reserves following standard procedures. Also, emerging adults mosquitoes, from same habitats as larval collections, had their wings measured for length as well as symmetry. The results indicated significant ($p<0.05$) variations in larval nutritional reserves among the four sites. Crude protein, in the fresh and dry larval samples, ranged from 15.06-19.00 and 51.86-70.37%, respectively; while lipids ranged from 0.09-1.91 and 0.13-10.65%, respectively. While, wing length differed significantly, wing symmetry varied only within narrow limits among the mosquito populations. The adult *Cx. p. pipiens* mosquitoes in the area are relatively large (wing length ranging between 3.45 ± 0.55 and 4.05 ± 0.62 mm). The ratio of the left and right wings ranged between 1.00: 0.99 and 1.00: 1.07 among the mosquito populations. Also, significant correlations (i.e., $r>0.70$) were established between larval nutritional components and wing symmetry in particular. These results were discussed from the perspective of fitness of *Cx. p. pipiens* populations for disease transmission in North central Nigeria. It is hoped that the findings of this study will go a long way in facilitating the development of informed complementary anti-larval strategies for integration into current mosquito vector control programs.

Key words: *Culex*, larva, spatial heterogeneity, teneral reserves, wing length, wing symmetry

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INTRODUCTION

Culex mosquitoes are the most abundant and wide-spread of Culicidae genera serving as vectors of human diseases (Brault, 2009). These mosquitoes pose serious threat to public health in many parts of the world, where they are responsible for the transmission of filariasis, elephantiasis, encephalitis, dengue fever, etc., These diseases constitute foremost public health challenges worldwide and exert heavy socio-economic burdens on people living in their areas of distribution (Braise *et al*., 2003; Awolola *et al*., 2004; Carter Centre, 2008). Filariasis alone, for example threatens the health of more than a billion people worldwide (WHO, 2004); almost 25 million men manifest filarial-genital infections (most commonly hydrocele) and about 15 million, mostly women, have lymphoedema or elephantiasis of the leg; with about 40 million disfigured and incapacitated by the disease (WHO, 2011).

According to Gafur (2004), the capacity of mosquitoes for disease transmission is greatly influenced by local environmental factors that condition their vectorial fitness, usually manifested in wing symmetry. Degree of wing symmetry in mosquitoes, on the other hand, reflects how well-formed internal organs and physiological processes are and thus their vector competence, i.e., ability to support pathogen development (Brault, 2009).

Also, mosquito wing length (a proxy for adult body size) has been demonstrated to correlate positively with mosquito fecundity (Blackmore and Lord, 2000; Armbruster and Hutchinson, 2002), adult life-span and blood meal success (Nasci, 1986; Renshaw *et al*., 1994; Ameneshewa and Service, 1996; Lyimo and Takken, 2003), as well as and vectorial capacity (Landry *et al*., 1988; Ameneshewa and Service, 1996). Adult body size of mosquitoes is largely dependent on larval dietary conditions, with respect to the quality and quantity of nutritional reserves acquired from the breeding habitat (Nasci, 1986; Timmermann and Briegel, 1996). More than 80% of immature development and accumulation of teneral reserve occur during the later larval instars. Such nutritional reserves are used for egg formation thus, translating into fecundity and by extension adult population density. If insufficient teneral reserves are acquired, pupation among surviving larvae may be delayed for as much as three weeks; even then the emerging adult mosquitoes tend to be smaller than the threshold required for significant vectorial capacity. This is so because in mosquitoes, newly emerged adults go through a teneral phase within 24 h post-emergence, during which covert anatomical, physiological and behavioural maturation take place, the quality of which are dictated by the quantity of teneral reserves. According to Briegel (1990a, b), adult body size is a reliable measure of larval teneral reserve conditions of mosquitoes and may be accurately indexed by cubic measure of wing length.

Therefore, the intensity of the transmission of mosquito-borne diseases in an area depends to a very large extent on the physiological fitness, reproductive potentials and adult longevity of the individual vector mosquito. In Nigeria, there is a dearth of information on these important determinants of mosquito vectorial capacity thus, making it difficult to put the epidemiology of mosquito-borne diseases in clear perspectives-a pre-requisite for the development of sustainable vector control strategies. To further compound this challenge; studies have revealed significant spatial variations in mosquito vectorial capacities (Brault, 2009; Gafur, 2004), which may be due to similar variations in their fitness and reproductive capacity; with attendant differential vector requirements in various localities. In order to fill these information gaps and provide baseline data for the development of sustainable locality-sensitive mosquito vector control strategies, this study was carried out to elucidate spatial variations in larval nutritional reserves, adult body size and wing symmetry of *Culex pipiens pipiens* mosquito populations in Minna area of North Central Nigeria.

MATERIALS AND METHODS

Description of study area and mosquito sampling locations: The study was carried out in and around Minna area of North Central Nigeria. Minna is the capital city of Niger state, 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. Minna enjoys a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20°C, 61.00% and 1334.00 mm, 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. Mosquito larvae collection was carried out at four different and widely separated localities; Gidan Kwano (A), Bosso (B), Maikunkele (C) and Chanchaga (D). Gidan Kwano (A), is a rural and sparsely populated area but with a large covering of trees and lowland grasses. It has no drainage system. The houses are mainly mud with only a few modern (Cement/Brick) houses. The inhabitants are mostly farmers, petty traders and students of the main campus of the Federal University of Technology, Minna.

The community is located about 13.8 miles (22.20 km) from Minna. Bosso (B), on the other hand, is an urban settlement about 4.25 miles (6.84 km) from Minna. It is densely populated, with the inhabitants mostly into civil service and trading. It is the seat of the Temporary campus of Federal University of Technology, Minna and hosts a numbers of small scale industries. The site is sparsely vegetated. Maikunkele (C), however, is less populated, located about 9.72 miles (15.64 km) from Minna. Its inhabitants are mainly farmers with few business men and civil servants. The site is dominated by thick vegetation. Chanchaga (D), though, densely populated, with good drainage system and low grass cover, is situated 5.51 miles (8.87 km) to the West of Minna city. It is the seat of the Niger state College of Education, an Army Barracks and hosts of other institutions and small scale industries.

Mosquito larval collection and laboratory rearing: Day old larvae of *Cx. p. pipiens* were collected from conventional mosquito breeding habitats in the four sites during the rainy season of 2011. The larvae were identified using the keys of Hopkin (1952). The larvae were reared in water from the respective original breeding habitats, following standard techniques for mosquito rearing and laboratory maintenance (Gerberg, 1970; Das *et al*., 2007; Olayemi and Ande, 2009).

Determination of adult wing length and symmetry: The wings of emerged adult mosquitoes were carefully detached and measured for length and symmetry following the techniques of Gafur (2004)

Determination of larval nutritional reserves: Fourth instar larval stage of *Cx. p. pipiens* were collected at regular intervals (weekly) from the four locations and cumulatively preserved separately to attain the required quantity for bio-chemical analysis. Collected samples were subjected to proximate analysis following standard procedures (AOAC, 1990).

Data analysis: Data collected were subjected to statistical analysis, using SPSS computer software (version 16.0). Mean wing length was separately determined for the left and right wings, by finding averages of length of wings from each side. Mean mosquito wing length per location, was calculated as the average of right and left wing lengths in the respective location. Wing symmetry was determined as the ratio of the difference between the left and right wings per location. Mean left and right wings lengths within location were compared for statistical significance using chi-square tests, while mean wing length (i.e., average of left and right lengths) among the locations were compared using ANOVA. The relationships between components of larval nutritional reserves and wing indices of vectorial fitness were established using Linear Coefficient Correlation.

RESULTS

Table 1 shows proximate composition of teneral reserves of 4th instar larvae of *Culex pipiens pipiens* populations in the study area. Generally, except for moisture content, the amounts of components analysed were significantly ($p<0.05$) higher in the dry matter than fresh. Moisture content in the dry matter, ash and lipid in the fresh samples of the larvae were not significantly ($p>0.05$) different among the 4 locations; the reverse was, however, the case for moisture in fresh matter, as well as ash and fat in the dry matter. Crude Protein (CP) was the only component that varied significantly in both the fresh and dry samples among the larval populations of the area. In the fresh samples, CP ranged from 15.06% in site A to 19.00% in C. However, in the dry state, the larvae from site A had the highest CP content (70.37%), closely followed by those from site D (68.11%). Site C produced the least protein-rich larvae of *Culex pipiens pipiens* (51.86%).

The lipid content of the dry larvae was abysmally low (0.13%) in the mosquitoes from site C but significantly highest in sites A and B (9.03 and 10.65%, respectively) (Table 1). However, the pattern of site-specific distribution of larval lipid content among the mosquito populations is very similar to that of crude protein (Fig. 1).

The mean Wing Length (WL), i.e., proxy for adult body size and Wing Symmetry (WS), i.e., proxy for adult vectorial fitness of *Culex pipiens pipiens* mosquito populations from the four study sites are presented in Table 2. WL of the mosquitoes varied significantly

Table 1: Larval teneral reserve (%) of *Culex pipiens pipiens* mosquito populations in Minna area of North Central Nigeria

	Moisture		Ash		Crude Protein		Lipids	
Larval collection site	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Gidan kwano (A)	79.61^{b*}	2.00^{a}	3.35^{a}	12.80^{b}	15.06^{a}	70.37^{c}	1.89^{a}	9.03^{c}
Bosso (B)	76.61^{a}	1.61^{a}	3.20^{a}	10.12^{b}	17.60^{ab}	64.35^{b}	1.91^{a}	10.65^{c}
Maikunkele (C)	79.04^{b}	1.73^{a}	3.64^{a}	11.12^{b}	19.00^{b}	51.86^{a}	0.99^{a}	0.13^{a}
Chanchaga (D)	75.61^{a}	1.99^{a}	2.44^{a}	7.95^{a}	16.85^{a}	68.11^{c}	1.37^{a}	6.87^{b}

Values followed by same superscript alphabets in a column are not significantly different at p = 0.05

Table 2: Mean wing length (mm) and symmetry of *Culex pipiens pipiens* mosquito populations in Minna area of North Central Nigeria

Larval collection site	No. of pairs of wings examined	Left wing	Right wing	Mean wing length	Wing symmetry (Lt wing:Rt wing)
Gidan kwano (A)	162	3.56 ± 0.60^{a}**	3.51 ± 0.50^{a}	$3.45\pm0.55_{a}$**	1.00: 0.99 (0.01)***
Bosso (B)	192	3.56 ± 1.04^{a}	3.81 ± 1.10^{b}	$3.59\pm1.07_{b}$	1.00: 1.07 (0.07)
Maikunkele (C)	200	3.53 ± 0.53^{a}	3.78 ± 0.53^{b}	$3.56\pm0.53_{b}$	1.00: 1.07 (0.07)
Chanchaga (D)	180	3.95 ± 0.64^{a}	4.15 ± 0.60^{b}	$4.05\pm0.62_{c}$	1.00: 1.05 (0.05)

Values followed by same superscript alphabets in a row are not significantly different at p = 0.05, **Values followed by same subscript alphabets in a column are not significantly different at p = 0.05, ***Values in parenthesis are differences in the ratio between left and right wings

Table 3: Correlation between wing variables and larval nutritional components of *Culex pipiens pipiens* mosquito populations in Minna area of north central Nigeria

Larval Nutritional Component	Wing length	Wing symmetry
Moisture (Fresh)	-0.8362*	-0.4395
Moisture (Dry)	0.3374	-0.8020*
Ash (Fresh)	-0.9117*	0.0644
Ash (Dry)	-0.9381*	-0.5071
Crude protein (Fresh)	0.0809	0.9306*
Crude protein (Dry)	0.2219	-0.7002*
Lipid (Fresh)	-0.3135	0.9653*
Lipid (Dry)	-0.0115	-0.3709

*Significant correlation

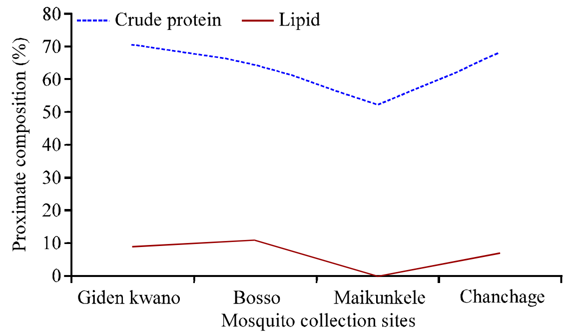

Fig. 1: Relative composition of crude protein and lipids in teneral reserves of *Culex pipiens pipiens* mosquito populations in Minna area of north central Nigeria

($p<0.05$) among the sites. Mosquitoes from site D were the largest (WL = 4.05±0.62 mm) while the smallest individuals were encountered in site A (WL = 3.45±0.55 mm). Except for site A, the left wings of the mosquitoes were significantly shorter than their right counterparts. On the other hand, however, the degree of symmetry between the right and left wings of the mosquitoes were more similar among the sites than wing length. The mosquitoes from site A had a near perfect wing symmetry (Right wing: Left wing = 1.00:0.99; difference = 0.01), followed by site D (Right wing:Left wing = 1.00:1.05; difference = 0.05), while the right and Left wings of mosquitoes from sites B and C were the most divergent (1.00:1.07; difference = 0.07).

The correlations between wing variables and larval nutritional components of the larvae are highlighted in Table 3. All the larval nutritional components investigated correlated significantly with at least one wing variable either in the fresh or dry state. Of particular importance are the significant correlations between the principal teneral reserve components (i.e., fresh protein and lipids; r = 0.9306 and 0.9653, respectively) and wing symmetry.

DISCUSSION

While certain proximate components of the larvae were not significantly different in a particular condition of matter (i.e., with respect to degree of dryness of larval specimens), the distribution of such components varied considerably in the opposite condition of matter among the mosquito populations. This observation suggests differences in solute concentrations of the haemocoel of the mosquito populations, probably, occasioned by significant differences in the physico-chemical properties of the larval breeding habitats. Earlier, Olayemi *et al*. (2010) reported significant differences in physico-chemical properties of conventional mosquito breeding habitats in the area; with attendant influence on species composition and relative abundance of mosquitoes in such habitats. Thus, significant differences in haemocoelic concentrations of *Cx. p. pipiens* populations in the area could be a physiological adaptation for surviving in widely varying larval habitats, thus, explaining the predominance of this mosquito species in the area (Olayemi *et al*., 2010).

Crude protein was the only teneral reserve component that varied significantly among the mosquito populations in both fresh and dry matter conditions. Also, while mosquito larvae from site C had the highest Crude Protein (CP) in fresh matter condition, those of site A were the most protein-rich when the dry matter samples were analysed. These results confirms the popular belief that nutrient protein reserve in mosquito populations, both intra-and interspecies, is highly dynamic and is often a function of the nutritional quality of larval breeding habitats. The late 4th instar larval stage of *Cx. p. pipiens* analysed for teneral reserve in this study should adequately reflect the nutritional quality of the larval breeding sites in the areas; as 80-90% of larval growth and teneral biosynthesis take place during this instar (Briegel, 2003). Thus, significant differences in crude protein content among *Cx. p. pipiens* populations in the study area may be due to disparity in the Nitrogen contents of the larval habitats. The nitrate content of conventional mosquito breeding sites in the area ranged from 0.50 mg L^{-1} in ponds to 20.70 mg L^{-1} in drains (Olayemi *et al*., 2010).

The distribution of lipid and CP among the mosquito populations followed more or less the same pattern. This finding probably indicates that the synthesis and reserves of lipid is equally important in the nutritional physiology of *Cx. p. pipiens* mosquitoes. Briegel (2003) documented the importance of lipids in yolk production, as well as, their energetic significance. Accordingly, the lipid reserve achieved during larval stage largely contributes to the reproductive potential of a female mosquito. Yet, the results of this study indicated a very low level of lipid reserve (0.13%) in the larval mosquitoes from site C, while those of other sites were not less than 6%; almost 11% in site B. This finding poses a great challenge to vitellogenesis, fecundity and ultimately population density of *Cx. p. pipiens*. in site C. To compensate for low teneral lipids, female mosquitoes often take sugar meals, which then enter the lipogenetic pathways, usually before blood meals are added (Briegel *et al*., 2001a, b). *Aedes vexans*, a highly successful large-bodied mosquito, even though low in teneral protein and lipids, must take sugar meal during the 1st week post adult emergence, or die within few days afterwards (Briegel *et al*., 2001a). On its parts, *Anopheles albimanus,* another species very poor in teneral reserves due to its small size, uses the first blood meal to synthesize maternal lipid and protein reserves and not for yolk synthesis, thus compensating for their low teneral reserves (Briegel, 1990a). Thus, it seems that the *Cx. p. pipiens* population in site C of the study area have evolved strategies for coping with its low teneral lipid reserves. However, physiological studies to elucidate such strategies are germane, to improve our understanding of the reproductive biology of the species, as well as, its ecological elasticity.

Wing length (WL), i.e., proxy for adult body size, also varied significantly among the four mosquito populations. This finding confirms the fact that larval teneral reserves, especially protein and lipids, definitely determine adult body size; and body size/WL is a reliable index of teneral reserve conditions. This view is consistent with that earlier expressed by Briegel (1990a, b) that total larval protein, significantly correlates linearly with body size, because at this stage protein represents mainly structural components; while, on the other hand, lipids may equally be linearly correlated with body size in ways similar to protein or may show steeply inclined exponential correlations.

The *Cx. p. pipiens* populations in the study area are generally composed of relatively large individuals, as their wing lengths were above 3.5 mm; wing length of 3 mm is generally regarded as threshold requirement for significant vectorial capacity in *Culex* mosquitoes. This finding has serious epidemiological implications regarding the vectorial capacity of *Cx. p. pipiens* in the study area. Bigger mosquitoes live longer and lay more eggs than their smaller counterparts (Takken *et al*., 1988), thus standing the species in good-stead as a formidable vector of *Culex* mosquito-borne human diseases in the area.

However, adult size of the *Cx. p. pipiens* mosquitoes varied significantly among the sites, with the largest mosquitoes collected from site D while, those from site A were the smallest. These results further support the likelihood of significant differences in the nutritional quality of larval breeding habitats among the 4 mosquito collection sites. Timmermann and Briegel (1998) opined that adult body size of mosquitoes is determined by the larval dietary conditions. This was demonstrated by Briegel (1990a, b) who routinely produced adult mosquitoes covering a wide spectrum of possible sizes in several mosquito species, by simply manipulating food supply. Briegel (1990b) observed that the requirement for mosquito larvae to live in stiff opposition between feeding in the water column and breathing atmospheric oxygen at the surface imposes feeding constraints that are reflected in significantly varied adult body size and teneral reserve. Thus, the significant variation in adult body size of *Cx. p. pipiens* mosquitoes among the 4 sites may mean heterogeneous disease transmission potentials of the species in the study area.

The left wings were significantly longer than their right counterpart in most of the mosquito populations investigated. The reason for this observation may go beyond the influence of environmental factors of the larval breeding habitats but, perhaps, endogenous factors especially genetics of the species may be responsible. However, the consistent longer left than right wing observed among the *Cx. p. pipiens* populations in the study area may negatively affect flight activity of the species, by limiting its manoeuvrability, thus reducing blood meal and mating success as well as escape from predators. There is, therefore, a need to elucidate the genetic bases of disproportionate wing length in *Cx. p. pipiens* populations as well as its implications regarding flight activities. To a large extent, the degree of right and left wing asymmetry among *Cx. p. pipiens* populations in the area varied only within narrow limits, probably, suggesting that the mosquitoes are subject to more or less same exogenous and endogenous stress and hence, equally fit as vectors of *Culex*-borne human diseases.

The nutritional components of *Cx. p. pipiens* larvae investigated in this study correlated significantly with the wing variables namely, wing length and symmetry. These results further confirm the strong relationships between mosquito larval nutrition and adult vectorial success indices. Of particular physiological importance is the strong correlation between the principal larval teneral reserves (i.e., Protein and Lipids) and wing symmetry, a proxy for the vectorial fitness of adult mosquitoes. Thus, in addition to the use of larviciding agents, mosquito vector control programs that include strategies targeted at disrupting the availability of food and larval feeding success in their breeding habitats may go a long way in reducing the vectorial fitness and hence, vectorial capacity of the emerging adult mosquitoes.

CONCLUSION

Spatial heterogeneity exists in larval teneral reserves of *Cx. p. pipiens* populations in Minna area of North central Nigeria and this was equally reflected in their varied influence on the indices of adult vectorial fitness of the mosquito species in the area. However, it is possible that populations of *Cx. p. pipiens* with very low teneral reserves have evolved special strategies for acquiring adequate lipids, post adult emergence, for the completion of vitellogenesis hence, sustaining threshold adult population density for disease transition. Though, strong correlations were established between larval nutritional reserve components and wing variables, the results of this study nevertheless hinted at genetic bases of adult vectorial fitness of Cx. *p. pipiens* mosquitoes. Therefore, further studies to elucidate the ecological elasticity and genetic bases of the vectorial fitness of *Cx. p. pipiens* are germane and will go a long way in improving our understanding of the epidemiological success of this important mosquito vector of human diseases. It is hoped that the findings of this study will facilitate the development of informed-complimentary strategies for integration into current mosquito vector control programs.

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