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Influence of *Plasmodium* Parasite Infection on Adult body size and Vectorial Fitness of Anopheline Vector Mosquitoes

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ABSTRACT*: The Study was designed to investigate the effect of Plasmodium parasite infection on Vectorial fitness of Anopheline mosquitoes. Adult mosquitoes were collected indoor using Pyrethrum Spray Catch (PSC). Morphological identification was carried out using a trinocular dissecting microscope with the aid of standard taxonomic keys. Dissection of the salivary gland was done to establish sporozoite infection. Wings were measured with an ocular micrometer from the apical notch to the axillary margin, excluding the wing fringe and used as proxy for body size. Data generated were analyzed using the SSPS software version 20.3 and excel package and processed using ANOVA, and Duncan multiple range test was used to compare their means. Findings revealed that Anopheline mosquitoes in Kontagora have a relatively similar body size as suggested by their wing length. Mean wing length (MWL) of Plasmodium infected and uninfected Anopheline mosquitoes do not varied significantly (P>0.05) across all the five sampling locations. The vectorial fitness of both Plasmodium infected and uninfected Anopheline mosquitoes were not significantly different (P>0.05) among the sampling locations. In this study, body size and vectorial fitness do not appeared to be predetermined factors for the infectivity of Anopheline mosquitoes as the proportion of Anopheline mosquitoes infected with sporozoites is independent of body size.*

KEYWORDS: P*lasmodium* Parasite, Infection, body size, Vectorial fitness, Anopheline mosquitoes.

INTRODUCTION

The terms vectorial capacity, competence, and fitness are often used interchangeably to describe the ability of a mosquito to serve as a disease vector. Vectorial capacity is defined quantitatively and is influenced by such variables as vector density and longevity as well as vector competence. Studies on the effect of *Plasmodium* infection on vector longevity are conflicting, with majority showing vector survival is unaffected, but some showing reduced vector survival (Nyasembe *et al* 2014). Estimates of vectorial capacity take into account all the environmental, behavioural,

cellular, and biochemical factors that influence association between a vector, the pathogen transmitted by the vector, and the vertebrate host to which the pathogen is transmitted (Beerntsen, 2000). Both behavioural and environmental factors can play a decisive role in determining vectorial fitness. For example, a particular mosquito species might be genetically and biochemically compatible for the complete development of a particular pathogen, but if this species does not coexist temporally and spatially with vertebrate host that harbours the parasite, or if the preferred blood source for this species does not include that vertebrate, the mosquito is not a suitable vector for the pathogen (Beerntsen, 2000). Lifetime fitness of adult female mosquitoes is influenced strongly by nutrition during larval development, the availability of quality blood meal and ambient condition (Takken *et al.,* 2013). Larval nutrition determines the size of adult mosquitoes upon emergence. The maintenance and transmission of the pathogens that cause malaria is without doubt absolutely dependent on the availability of highly efficient and competent mosquito vectors (Breda *et al.,* 2000). The high vectorial success of *Anopheles* mosquitoes is however, largely due to its superior reproductive capacity and biologic fitness, relative to the secondary *Anopheline* mosquito vector species (Bockarie *et al*., 1994).

Mosquitoes, like all organisms, are under constant threat of infection (Hillyer *et al.,* 2010). The act of blood feeding often exposes mosquitoes to blood borne pathogens. Upon ingestion of an infectious blood meal, the parasite begins sporogonic development (Sinden, Alavi and Raine, 2004). During this time the parasite causes damage to the mosquito (Hurd and Carter, 2004) and triggers an immune response against it (Michael and Kafatos, 2005; Dong *et al.,* 2006). (Ferguson and Read, 2002). Body size is a pivotal trait for mosquitoes, because it has been related to survival, blood feeding behavior, reproductive success and vectorial fitness. The best measure of body size is assumed to be dry weight; therefore, weight is used in many studies involving association with body size (Carron, 2007). However, the weight of an adult mosquito varies considerably and depends on whether the mosquito has recently had blood or sugar meal. For example, mosquito weight can sometimes give unreliable results due to different factors such as gravidity or recent intake of blood meal (Carron, 2007). To circumvent this problem, many researchers have used wing length as an indicator of body size. Wing length which is correlated with dry weight can be used as a proxy for body mass (Takken *et al.,* 2011). Body size therefore confers better fitness to mosquitoes in a natural population. Malaria transmission can segregate over very small geographical scales and could influence vector and parasite fitness when they interact with different local populations/species (Joy, *et al.,* 2008). Variability of body size within mosquito population as a factor affecting malaria transmission has received only little attention. Information about body size of Anopheline mosquitoes in Kontagora and its influence on malaria parasite infection and transmission is grossly inadequate. Hence, the desire to investigate the influence of *Plasmodium* parasite infection on body size and vectorial fitness of Anopheline vector population.

MATERIALS AND METHODS

Study area and sampling locations

Kontagora is a major town on the south bank of Kontagora River in North West Niger State. The town is situated at 10.4^0 North latitude, 5.47^0 East longitude and 335 meters elevation above sea level with an estimated population of over 200,000 inhabitants. The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20^0C, 61.00% and 1334.00mm, respectively. The climate presents two distinct seasons; 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. Adult mosquito samples were collected from five sampling sites located in Kwangwara, Sabon gari, Tudun wada, Dadin Kowa and Usubu areas of Kontagora.

Adult mosquito collection and preservation

Indoor resting mosquitoes were collected using the Pyrethrum Spray Catch (PSC) between the hours 06:00 and 09:00 am in the study areas. Food items were covered properly and moveable furniture were taken care of before spraying. Large white sheets of cloths was spread wall to wall to cover the floors of the room while all doors and windows were shut. All cracks and openings in walls were stocked with rag papers to prevent mosquitoes from escaping. After about 20 minutes, the spread cloths were carefully folded starting from the corners. Knock down mosquitoes were collected with forceps into a damp petri dish.

Identification

Morphological identification was carried out using a trinocular dissecting microscope (Amscope SZMT2/MU100010APTINA COLOR CMOS) with the aid of standard keys (Gillies and Coetzee, 1987; Gillies and De Meillon, 1968).The mosquitoes were identified using the gross morphology of the species, external morphology of the head, mouthparts, antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments (Gillet and Smith, 1972).

Dissection of salivary gland for determination of sporozoite infection

Dissection of the salivary gland was done according to the methods described by Looker and Taylor-Robinson (2004). This was intended to incriminate mosquito vectors and establish Sporozoite rates. The anterior part of the same mosquito was placed on a slide with the head pointing to the right. A drop of saline was added to keep the specimen fresh. Meanwhile, the left dissecting needle was placed gently on the thorax, just below the region where the glands lie. The right needle was pulled towards the right direction to bring out the head with the salivary glands attached. Some salivary glands may not come out with the head of the mosquito but these were located by carefully teasing the lower part of the thorax. The glands were detached from the head and then placed on another microscope slide with little drop of saline and covered with a cover

slip. A gentle pressure was exerted on the cover slip to rupture the gland tissues freeing the sporozoites into solution. The sporozoites were seen under a microscope (if present) and identified as minute needle like forms.

Wing measurements

Wing length used as a proxy for body size was determined as previously described (Schneider *et al*., 2007). Wings will be measured with an ocular micrometer from the apical notch to the axillary margin, excluding the wing fringe. To minimize measurement errors, all wing lengths was determined by a single researcher. Both left and right wings of individual adult mosquitoes were removed carefully with forceps and mounted on a microscope glass slide. The wings were measured using calibrated dissecting microscope following the techniques of Gafur (2004), and the differences between mean right and left wings were determined as Fluctuating Asymmetry.

Data analysis

Data generated were analyzed using the SSPS software version 20.3 and excel package. Mean wing Length (MWL) and Fluctuating Asymmetry (FA) of *Plasmodium* infected and uninfected *Anopheline* mosquitoes were processed using ANOVA, and Duncan multiple range test was used to compare their means.

RESULTS AND DISCUSSIONS

Results

The mean wing length of Anopheline mosquitoes collected from various sampling locations were measured to ascertain their body size and the data is presented in Table 1. Findings revealed that Anopheline mosquitoes in Kontagora have a relatively similar body size as suggested by their wing length. Although the mean wing length (MWL) for *Plasmodium* infected mosquitoes in Dadin kowa were longer with a mean value of (3.19±0.04). But those encountered at Kwangwara and Sabon gari have a mean wing length (MWL) of (3.15±0.03) and (3.14±0.03) respectively. Tudun wada and Usubu areas recorded mean wing length of (3.14±0.03) and (3.14±0.04) respectively. However, the mean wing length (MWL) of *Plasmodium* infected and uninfected Anopheline mosquitoes do not varied significantly (P>0.05) across all the five sampling locations.

Table 1: Mean wing length (MWL) in (mm) (mean ± SE) of *Plasmodium* infected and uninfected Anopheline mosquitoes

Location	Infected	Uninfected
Kwangwara	3.15 ± 0.03^a	3.13 ± 0.03^a
Tudun wada	3.14 ± 0.03^a	3.17 ± 0.04^a
Sabon gari	3.15 ± 0.10^a	3.14 ± 0.03^a
Dadin kowa	3.19 ± 0.04^a	3.15 ± 0.03^a
Usubu	3.14 ± 0.04^a	3.13 ± 0.10^a
Aggregate mean	3.15 ± 0.05^a	3.14 ± 0.05^a

Fluctuating Asymmetry (FA) was determined as the difference between the length of right and left wings. Table 2 provided detailed information on Fluctuating Asymmetry (FA) of wings (Mean ±SE) of both *Plasmodium* infected and uninfected Anopheline mosquitoes. All categories of *Anopheline* mosquitoes (i.e. infected and uninfected) recorded a very minimal difference in the mean wing length of left and right wings. A Fluctuating Asymmetry (FA) of less than 1.0mm was recorded in both *Plasmodium* infected and uninfected Anopheline mosquitoes across the five sampling locations. The vectorial fitness of both *Plasmodium* infected and uninfected Anopheline mosquitoes were not significantly different (P>0.05) among the sampling locations.

Table 2: Fluctuating asymmetry (FA) in (mm) of wings (mean ± SE) of *Plasmodium* infected and uninfected Anopheline mosquitoes

Location	Infected	Uninfected
Kwangwara	0.08 ± 0.03^{a}	0.07 ± 0.04^{a}
Tudun wada	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
Sabon gari	0.06 ± 0.02^{a}	0.07 ± 0.02^{a}
Dadin kowa	0.01 ± 0.03^{a}	0.01 ± 0.01^{a}
Usubu	0.04 ± 0.08^{a}	0.06 ± 0.03^{b}
Aggregate mean	0.09 ± 0.03^{a}	0.04 ± 0.02^{a}

DISCUSSIONS

This study showed no positive relationship in both the body size estimated by wing length, vectorial fitness estimated by fluctuating asymmetry of wings and *Plasmodium* parasite infection. This is because no significant variation was observed in the wing length and fluctuating asymmetry of *Plasmodium* infected and uninfected Anopheline vectors. Wing length therefore do not appeared to be a predetermined factor for the infectivity of *Anopheline* mosquitoes in Kontagora. Although, all the five sampling locations were ecologically similar providing similar nutritional richness for immature aquatic stage of Anophelines. Perhaps, this might further explain the lack of variability in the wing length and fluctuating asymmetry of Anopheline mosquitoes in the study area. The aggregate mean wing length MWL of infected and uninfected Anopheline mosquitoes in this study was 3.15 ± 0.05^{a} and 3.14 ± 0.05^{a} respectively. This shows no significant variation in body size between *Plasmodium* infected and uninfected Anophelines. Therefore, the population of Anopheline mosquitoes infected with *Plasmodium* parasite was independent of body size.

The results of this study was corroborated by Barreaux *et al* (2016) who reported that wing length had no effect on the probability of habouring sporozoites in salivary glands of Anopheline mosquitoes. `Lyimo and Koella (1992) had earlier reported a situation where the proportion of mosquitoes infected with oocyst was independent of size. They further reported that the proportion of mosquitoes with sporozoites increased from (19.5%) in the smallest size class mosquitoes to (41.1%) in the intermediate size class and dropped again to (7.1%) in the largest mosquitoes. This makes it difficult to conclude that such variability in infection is a function of body size. However,

Selective pressures exerted by the parasite on its host and vice versa are expected to drive evolution for each to maximize its fitness (Haris *et al*., 2012). Therefore, other physiological factors besides *Plasmodium* infection can influence the size and vectorial fitness of *Anopheline* mosquitoes. The impact that the malaria parasite has on the body size and vectorial fitness of *Anopheles* mosquitoes is likely dependent on parasite-mosquito species combinations and environmental conditions (Aboagye-Antwi *et al*., 2010). In another study, Mwangargi *et al* (2004) reported an MWL of 2.94m with no site to site variation in body size and no significant variation sporozoite infected and uninfected *Anopheles gambiae* s.s along the Kenyan coast. Furthermore, the population of infected mosquitoes reported in the study was independent of size. However the number of oocysts harbored by infected mosquitoes increased with size of the mosquitoes. Since mosquito body size has been associated with blood feeding behaviour, therefore, blood feeding preference can be a key factor for *Plasmodium* parasite infection of Anopheline mosquitoes. For instance, *Anopheles* species that has high preference for non-human blood feeding may record low *Plasmodium* parasite infection rate and the quality of blood meal will influence the mosquito body size. In period of stress smaller larvae will not survive and hence adult size at emergence will be skewed. This is seen to happen in tree-hole mosquitoes as was the case for the univoltine temperate mosquito, *Aedes cantans (*Renshaw *et al*., 1994). It might be expected that mosquitoes occupying temporary, resource-limited habitat as do *Anopheles gambiae* would suffer such stress. Nevertheless, despite high mortality among larvae, wing length distributions of both males and females *Anopheles gambiae* from Tanzania (Lyimo and Takken, 1993) and Sa'o Tome are close to normal. The size of the emerging adult is of importance as larger females survive longer and greater fecundity (Takken *et al.,* 1998). Moreover smaller and virgin females requires a second or third blood meal in order to develop mature eggs, prolonging the time of their first oviposition (Lyimo and Takken, 1993) and suggesting that adult size of mosquitoes therefore affects host seeking behavior and parasite infectivity. Different habitat characteristics and productivity may contribute to different sizes of Anopheline mosquitoes. However, variation in body size of Anopheline mosquitoes did not seem to correlate with *Plasmodium* parasite infection.

CONCLUSIONS

In this study, body size and vectorial fitness do not appeared to be predetermined factors for the infectivity of *Anopheline* mosquitoes. This is because the study established no positive relationship between *Plasmodium* parasite infection, wing length and fluctuating asymmetry of *Anopheline* mosquitoes in Kontagora. Therefore, the proportion of *Anopheline* mosquitoes infected with sporozoites is independent of body size.

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