Comparative account of energy reserves in four co-occurring mosquito species in Kolkata, India (Diptera: Culicidae)

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ABSTRACT. Energy reserves in mosquitoes are an indicator of fitness, linking larval effort in resource acquisition with adult survival and fecundity. In other words, life history strategies and disease transmission potential can be related to the amount of energy reserves. The energy reserves of four mosquitoes – *Aedes aegypti*, *Aedes albopictus*, *Armigeres subalbatus* and *Culex quinquefasciatus* (Diptera: Culicidae) – were calculated to justify species-specific differences in their life history strategies. Following repeated sampling of pupae from the respective larval habitats, the glycogen, sugar and lipid contents of individual mosquitoes were assessed and corroborated with pupal weight and adult wing length. Discriminant function analysis was used to acquire an initial reflection of the differences of the parameters among the sex and species of the mosquitoes considered in the study. Using logistic regression and ANOVA, the effects of species and sex as contributors to variations in energy reserves could be established. The results indicated that for all the mosquitoes, sex-specific differences were prominent with reference to the energy reserves. Species-specific differences in energy reserves reflect differences in resource acquisition and assimilation in the tissues, and thus the differences in the life history strategies of these four species.

KEY WORDS: energy reserves, mosquito, *Aedes*, *Armigeres*, *Culex*, sexual dimorphism, multivariate analysis.

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INTRODUCTION

In mosquitoes, resource acquisition in the larval stages determines the energy reserves in adults. Energy reserves can be regarded as a life history trait owing to their correspondence with the longevity and fecundity of an individual mosquito (MOSTOWY & FOSTER 2004, BARGIELOWSKI et al. 2012, MAIGA et al. 2012, KAUFMANN et al. 2013). Body sizes (pupal weight, adult weight, wing length etc.) of mosquitoes are also dependent on larval feeding, so the food resources of larval habitats play a significant role in the mosquito life cycle (ARRIVILLAGA & BARRERA 2004, MOGI 2010) and mosquito abundance (BANERJEE et al. 2015). For a particular mosquito species, the larger the size of an individual, the higher the content of protein, glycogen and lipid reserves at emergence, which influences the fecundity and longevity and thereby contributes to the fitness of the mosquito (NASCI 1986, TAKKEN et al. 1998, BRIEGEL 2003). Small, nutritionally deprived mosquito larvae have lower energy reserves than larger ones, as a consequence of which smaller female mosquitoes need to take more blood meals for egg maturation during the adult stage (BRIEGEL 1990, BRIEGEL & TIMMERMANN 2001). This has been demonstrated in *Aedes aegypti* (LINNAEUS, 1762) (GRIMSTAD & WALKER 1991, NAKSATHIT et al. 1999), *Culex pipiens pallens* (LINNAEUS, 1758) (SHIN et al. 2012) and GILES, 1902 (TAKKEN et al. 1998). Energy reserves can thus be considered crucial indicators of larval efforts towards resource acquisition and assimilation in the adult body size, and also determinants of longevity and fecundity.

The disease transmission potential of vector mosquitoes is linked with life history traits like body size and longevity. Empirical studies have shown that vector competence for arboviral pathogens corresponds to the body size and energy reserves in an individual mosquito (TAKAHASHI 1976, BAQAR et al. 1980, PAULSON & HAWLEY 1991, SUMANOCHITRAPON et al. 1998, MUTURI et al. 2011). Species-specific variations in behavioural and life history strategies have been observed for different vector mosquitoes, which accounts for the varying levels of vector competence (RICHARDS et al. 2012, TABACHNICK 2013, TSURIM et al. 2013). This is evident from the pattern of transmission of different mosquito-borne diseases like malaria, filariasis, Japanese encephalitis and dengue. The oviposition habitat preferences and the developmental pattern of vector mosquitoes may result in differences in life history traits and thus energy reserves. As a consequence of the different strategies for larval development, including resource acquisition, energy reserves in different mosquitoes may vary. Even if mosquitoes develop in the same habitats, their physiological, genetic and morphological features may lead to differences in the developmental pattern and thus resource acquisition and energy reserves. The resource quality of larval habitats can also contribute to the differences in the energy reserves of mosquitoes. For instance, mosquitoes developing in sewage drains vary in larval

development and consequently in energy reserves, in contrast to those developing in phytotelmata and smaller container habitats (BANERJEE et al. 2015). The hypothesis of species-specific differences in energy reserves is explored in the present study using four different Culicine mosquito species, namely *Aedes aegypti*, *Aedes albopictus* (SKUSE, 1894), *Armigeres subalbatus* (COQUILLET, 1898) and *Culex quinquefasciatus* SAY, 1823 (Diptera: Culicidae). In Kolkata, India, the records of the larval habitats of these mosquitoes indicate that *Culex quinquefasciatus* and *Armigeres subalbatus* prefer larger larval habitats as breeding sites, while *Aedes aegypti* and *A. albopictus* prefer smaller container habitats (ADITYA et al. 2009, ADITYA & SAHA 2013, BANERJEE et al. 2015). Although field observations provide an impression of the differences in the life history strategies and body sizes of these four mosquitoes, comparative studies of the energy reserves and body size correlates have been made separately, mostly on the basis of inbred laboratory populations (TAKKEN et al. 1998, BRIEGEL 2003, SHIN et al. 2012). Thus, we hypothesize that, if energy reserves are a species-invariant property, the correspondence between body size and energy reserves will remain similar in all the mosquitoes concerned. If it varies with the species, however, differences in life history strategies can be deduced, which in turn may help understand the variations in the transmission of different vector borne diseases.

MATERIAL AND METHODS

Study sites

Larval sampling was carried out in six broad areas of Kolkata and surroundings: Belghoria (22°39'56.01" N, 88°22'45.26" E), Kasba (22°31'3.42" N, 88°23'2.58" E), Garia (22°27'46.61" N, 88°23'48.31" E), Park Circus (22°32'16.39" N, 88°22'5.69" E), Dankuni (22°40'51.00" N, 88°17'34.55" E) and Howrah (22°35'44.77" N, 88°15'49.10" E). The immature stages of four mosquito species – *Armigeres subalbatus*, *Culex quinquefasciatus*, *Aedes aegypti*, and *Aedes albopictus* – were randomly collected from at least 20 larval habitats of each site using standard sampling methods (BLACKMORE & LORD 2000, WHO 2003, ADITYA et al. 2009, BANERJEE et al. 2010) from July to September of 2011 and 2012. The collection times and the study period were selected to coincide with the abundance of all four mosquito species. At least 20 prospective larval habitats were surveyed in each month for collections of immature mosquitoes. Sewage drains and storm water pits were regarded as larval habitats for the mosquitoes *Armigeres subalbatus* and *Culex quinquefasciatus* (ADITYA et al. 2009, ADITYA & SAHA 2013), whereas plastic and porcelain containers were considered for the mosquitoes *Aedes aegypti* and *A. albopictus* (BANERJEE et al. 2010, 2015). Sampling was continued for different time periods from the

same geographical locations, such that the samples were random and interspersed in compliance with the norms of replication (HURLBERT 1984).

Larval and pupal sampling and rearing to adult stages

The pupae and larvae from each positive habitat were collected randomly and placed in sample containers (100 ml sample container Tarsons®, India) and brought to the laboratory. There the specimens were placed in plastic trays (15x11x3 cm) for segregation of pupa and larva, identification and data recording. The pupal weight was measured (wet weight up to the nearest 0.1 mg using an Adam equipment analytical balance, ADA 71/L, UK) in mg and each pupa was placed in a glass vial (with 5mL of deionized water) individually and allowed to emerge as an adult at room temperature (27-30°C). The larger larvae (late IV instar stages), i.e. those that pupated without any additional food resources within 2 hours of collection, were also considered. The sex and species of the adults were identified on the basis of the relevant keys (CHRISTOPHER 1933, BARRAUD 1934, NAGPAL et al. 2005). Following eclosion, the water in the glass vials was removed and the glass vials along with the adult mosquitoes were stored at -20°C for 2 hours to kill the insects. Then the vials were placed in a hot air oven with a temperature of 40°C maintained for 48 hours to dry the samples, following the protocol used elsewhere (TELANG et al. 2006, 2007). This process was adopted for adults emerging within a 2 hour time frame; the remainder were continuously monitored for eclosion to adult stage. In no instance were adult mosquitoes allowed to feed on a sugar solution. One of the wings was then separated from each individual and its length measured to the nearest 0.1 mm using a dissecting stereo microscope (Olympus® SZX, Olympus Corporation, Tokyo, Japan) fitted with a graduated eyepiece (Erma®, Japan) (appropriate magnification, scaling and conversion of the eyepiece to mm was done). The dead mosquitoes were then subjected to biochemical analyses to estimate the energy reserves in each species. Adult eclosion varied in accordance with the numbers of each species collected, and the energy reserves were estimated accordingly.

Estimation of glycogen, sugar and lipid content

The glycogen, sugar and lipid contents of individual adults of four mosquito species (*Armigeres subalbatus*, *Culex quinquefasciatus*, *Aedes aegypti* and *A. albopictus*) were estimated following standard methods (VAN HANDEL 1965, 1985a, 1985b). Dead and dried mosquitoes were individually homogenized in 0.2 ml 2% sodium sulphate and a few drops of methanol in a 15×155 mm glass tube. This solution was vortexed (Remi CM 101, India) vigorously, after which a mixture of methanol and chloroform (1:1) was added to it. The tissues were crushed and then stirred. The mixture was centrifuged (HERMLE® Z323K,

USA) at 2000 RPM for 20 min. The supernatant was transferred into a 15×155 mm glass test tube (marked up to 10 ml, graduated at 0.1 ml) for the sugar assay, while the precipitate was left for the glycogen and lipid assays. The test tubes containing sugar were heated to evaporate the solution in it to 0.1-0.2 ml. Anthrone solution was added (up to the 5 ml mark), stirred and heated in a water bath for 17 min. Following this the test tubes were cooled in an ice box and then stirred. The absorbance of the resultant solution was measured at 625 nm in a spectrophotometer (SHIMADZU® UV-17000, India) and compared with glucose standards.

To the precipitate left earlier, 0.5 ml chloroform and methanol were added and the whole gently shaken. The mixture was centrifuged at 2000 RPM for 10 min using a HERMLE® Z323K, USA, centrifuge, yielding a supernatant (containing glycogen) and precipitate (lipid). The supernatant containing glycogen was collected in separate test tubes (15×155 mm, marked up to 10 ml, graduated at 0.1 ml) and then heated well to evaporate the solution. Next, anthrone solution was added to it (up to the 5 ml mark), stirred and heated in a water-bath for 17 min. It was then allowed to cool in an icebox and stirred. The absorbance of the resultant solution was taken at 625 nm using spectrophotometer (SHIMADZU® UV-17000, India) and compared with glucose standards.

For estimating the lipid content, the precipitate was dissolved in a mixture of methanol: chloroform (1:1). The test tubes (15×155 mm, marked up to 10 ml, graduated at 0.1 ml) were put in a water bath for evaporation and 0.2 ml concentrated sulphuric acid added. Then they were cooled in an icebox and vanilin was added up to the 5 ml mark. The solution was stirred and a reddish colour allowed to develop within 5 min. The absorbance was measured at 525 nm using a spectrophotometer (SHIMADZU® UV-17000, India) and compared with the lipid standard. All the standard curves for sugar, glycogen and lipids were prepared with 10, 20, 30, 40, 50 ml concentrations of glucose and lipid solution (mg/ml) using the same reagents, and the same treatment was applied to the mosquito tissues. A schematic diagram of the experimental setup is shown in Fig. 1.

Data analysis

To analyse the sex- and species-specific variations between the energy reserves and the pupal weight and wing length [life history traits i.e. pupal weight, PW (in mg), wing length, WL (in mm), glycogen content, GLY (in mg), sugar content, SUG (in mg) and lipid content, LIP (in mg)], the data were subjected to two-way factorial ANOVA, taking sex and species as explanatory variables. The sex- and species-specific differences in the mosquitoes were further expressed by means of discriminant function analysis (MANLY 1994), in which the relative differences among the eight groups (4 species and 2 sexes) were portrayed using features like pupal weight, wing length, and three levels of energy

reserves. The results would enable justifying the difference, if any, among the four species considered in the study.

Fig. 1. Outline of experimental design to estimate the relation between life history traits and energy reserves. A – *Armigeres subalbatus*, B – *Culex quiquefasciatus*, C – *Aedes aegypti*, D – *A. albopictus*.

A logistic regression complying with the binomial generalized linear model with logit link was employed to comment on the sex- and species-specific variations in the life history traits of the four mosquito species using sex and species as explanatory variables. In the logistic regression, a particular life history trait (response variable) is assumed to follow a binomial (n, p) distribution with n replicates (sampling efforts) for each level of explanatory variables (the levels of the explanatory variables: Sex-2, Species-4). The linear combination of the explanatory variables is represented through the probability parameter p. A weighted binary function with logit link was employed and the parameters were estimated by maximum likelihood using XLSTAT software (ADDIN SOFT 2010). The equation is represented as a life history trait (y) = $1/(1 + \exp(-(a + b_1x_1 - b_2x_2)))$, where x_1 is species, x_2 is sex. A Chi square value (Wald's Chi-square) was used to deduce the significant contribution of the explanatory variables. All statistical analyses were done following ZAR (1999).

The difference between the male and female adult mosquitoes with respect to glycogen, sugar and lipid contents were highlighted by the degree of sexual dimorphism (SHARMILA BHARATHI et al. 2004) using the following formula:

$$\text{Degree of sexual dimorphism (DD)} = (ER_F - ER_M) / [(ER_F + ER_M)/2]$$

where ER_F = energy reserve of the i th female and ER_M = energy reserve of the i th male.

Prior to this, the data on the glycogen, sugar and lipid contents of individuals were weighted and scaled separately by taking the corresponding pupal weight as the denominator. The values were then arranged in ascending order for the two sexes, in accordance with the respective pupal weight, and DD was calculated for glycogen, sugar and lipid separately, using the above formula. The difference between males and females was judged to be significant by the deviation from 0 (no difference) (ZAR 1999) using the one-tailed t-test for each of the four mosquito species.

RESULTS

The pupal weight and wing length of the mosquitoes varied with the species and sex, with *Armigeres subalbatus* and *Culex quinquefasciatus* exhibiting higher values than the *Aedes* mosquitoes. Irrespective of the mosquito species, the mean values of the pupal weight and the wing length of the males were smaller than in the females (Table 1). The energy reserves of the four mosquito species, when expressed as a function of unit pupal weight, were found to vary according to species, but no fixed pattern for the sex-specific variation was found (Fig. 2). The results of the two-way factorial ANOVA indicate that

Table 1. The values (Range, Mean $\pm$ SE) of the pupal weight (PW), adult wing length (WL) and energy reserves (Sugar, SUG, Glycogen, GLY, and Lipid, LIP,) of the four mosquito species collected from larval habitats in Kolkata, India. Sp. – Species: *Armigeres subalbatus* (Asu), *Culex quinquefasciatus* (Cqu), *Aedes aegypti* (Aae) and *A. albopictus* (Aal).

Sp.	Sex	PW [mg]	WL [mm]	GLY [mg]	SUG [mg]	LIP [mg]
Asu	♀ N=77	4.50 – 6.20	4.690 – 5.020	1.500 – 1.600	0.216 – 0.289	0.214 – 0.333
		5.62 $\pm$ 0.06	4.880 $\pm$ 0.009	1.560 $\pm$ 0.003	0.239 $\pm$ 0.002	0.293 $\pm$ 0.004
	♂ N=77	4.50 – 6.20	4.670 – 5.000	1.500 – 1.600	0.213 – 0.280	0.199 – 0.321
		5.62 $\pm$ 0.06	4.860 $\pm$ 0.009	1.55 $\pm$ 0.002	0.234 $\pm$ 0.002	0.280 $\pm$ 0.004
Cqu	♀ N=102	2.00 – 2.30	4.590 – 4.920	0.230 – 0.260	1.206 – 1.370	0.010 – 0.050
		2.12 $\pm$ 0.01	4.830 $\pm$ 0.010	0.250 $\pm$.0010	1.360 $\pm$ 0.003	0.030 $\pm$ 0.001
	♂ N=102	1.70 – 2.10	4.590 – 4.920	0.230 – 0.260	1.120 – 1.370	0.010 – 0.040
		1.85 $\pm$ 0.01	4.790 $\pm$ 0.010	0.240 $\pm$ 0.001	1.240 $\pm$ 0.010	0.028 $\pm$ 0.001
Aae	♀ N=100	2.30 – 4.70	2.290 – 6.710	0.128 – 0.618	0.042 – 0.816	0.021 – 0.062
		3.82 $\pm$ 0.06	3.080 $\pm$ 0.060	0.223 $\pm$ 0.011	0.155 $\pm$ 0.010	0.044 $\pm$ 0.001
	♂ N=100	0.50 – 3.42	1.800 – 3.390	0.027 – 0.611	0.038 – 0.507	0.015 – 0.054
		1.98 $\pm$ 0.07	2.450 $\pm$ 0.030	0.221 $\pm$ 0.012	0.149 $\pm$ 0.007	0.038 $\pm$ 0.001
Aal	♀ N=100	1.80 – 4.30	2.150 – 3.600	0.040 – 0.568	0.036 – 0.437	0.019 – 0.058
		2.60 $\pm$ 0.06	2.760 $\pm$ 0.003	0.160 $\pm$ 0.012	0.134 $\pm$ 0.005	0.042 $\pm$ 0.001
	♂ N=100	0.50 – 3.00	1.120 – 2.440	0.053 – 0.571	0.018 – 0.494	0.008 – 0.053
		1.86 $\pm$ 0.06	2.070 $\pm$ 0.010	0.158 $\pm$ 0.009	0.133 $\pm$ 0.007	0.031 $\pm$ 0.001

pupal weight shows both species- and sex-specific variations but that wing length varied significantly only with the species. Whereas energy reserves, particularly of sugar, varied significantly for the four different species, both glycogen and lipid reserves showed both species- and sex-specific variation (Table 2). The results of the GLM resulted in significant differences in the pupal weight as explained by the sex and species of the mosquitoes, whereas the wing length displayed species-specific variation. The significant contribution of sex and species were observed across the values of Wald's Chi-square values (Table 3). The results of the discriminant function analysis highlighted the differences among the mosquitoes and the two sexes with considerable resolution (Fig. 3). The first two extracted factors were able to explain 93% of the variation in the data on pupal weight, wing length and energy reserves. Significant values of the Mahalanobis distance matrix among the species and sex of the mosquitoes were manifested owing to the differences in the life history traits and energy reserves. Irrespective of the sexes, the differences between *Culex quinquefasciatus* and *Armigeres subalbatus* were greater than those between *Aedes aegypti* and *A. albopictus*. The results of the discriminant function analysis as well as factorial ANOVA indicate that the four mosquito species differed significantly in terms of pupal weight, wing length and energy reserves. Comparison of the degree of sexual dimorphism indicated that the female mosquitoes were larger than the males (Fig. 4). As a consequence,

the extent of differences between the two sexes was not pronounced in case of the energy reserves weighed according to pupal weight. Since the female mosquitoes were heavier in size, the contribution per unit biomass of pupa reduced the value of the energy reserves to a greater extent than in their male counterparts.

Table 2. The results of two-way factorial ANOVA using the sex and species of the mosquitoes as the source of variations for the response variables pupal weight (PW), wing length (WL), glycogen (GLY), sugar (SUG) and lipid (LIP) reserves. The values in bold represent significance at $P < 0.05$ level.

Source	DF	Sum of squares	Mean squares	F
PW [mm]				
Species	3	1390.135	463.378	1802.472
Sex	1	109.108	109.108	424.415
Species*Sex	3	92.854	30.951	120.396
Error	750	192.810	0.257	
Total	757	1784.907		
WL [mm]				
Species	3	969.532	323.177	4104.991
Sex	1	0.000316	0.000316	0.004
Species*Sex	3	43.824	14.608	185.549
Error	750	59.046	0.079	
Total	757	1072.401		
SUG [mg]				
Species	3	1.870	0.623	198.147
Sex	1	0.002	0.002	0.730
Species*Sex	3	0.011	0.004	1.176
Error	750	2.360	0.003	
Total	757	4.243		
GLY [mg]				
Species	3	286.664	95.555	13147.700
Sex	1	0.202	0.202	27.795
Species*Sex	3	0.490	0.163	22.494
Error	750	5.451	0.007	
Total	757	292.807		
LIP [mg]				
Species	3	7.732	2.577	7671.371
Sex	1	0.011	0.011	31.733
Species*Sex	3	0.004	0.001	3.961
Error	750	0.252	0.000336	
Total	757	7.999		

Table 3. The results of the logistic regression (binomial generalized linear model) with logit link to deduce the variations in the life history traits and energy reserves of the four mosquito species, using sex and species as explanatory variables. Species-specific variations were consistent for all the response variables considered.

$$\begin{aligned} \text{PW} &= 1 / (1 + \exp(-(-5.58 - 0.28 * \text{Species} - 0.25 * \text{Sex}))) \\ \text{WL} &= 1 / (1 + \exp(-(-5.98 - 0.27 * \text{Species} - 0.0003 * \text{Sex}))) \\ \text{SUG} &= 1 / (1 + \exp(-(-6.05 - 0.22 * \text{Species} - 0.018 * \text{Sex}))) \\ \text{GLY} &= 1 / (1 + \exp(-(-4.91 - 0.77 * \text{Species} - 0.043 * \text{Sex}))) \\ \text{LIP} &= 1 / (1 + \exp(-(-4.63 - 0.88 * \text{Species} - 0.086 * \text{Sex}))) \end{aligned}$$

Variable	Factor	Intercept	Species	Sex
PW	Value	-5.584	-0.278	-0.252
	SE	0.078	0.020	0.042
	Wald χ^2	5148.091	202.152	35.863
	Pr > χ^2	< 0.0001	< 0.0001	< 0.0001
WL	Value	-5.980	-0.267	0.0003
	SE	0.073	0.018	0.038
	Wald χ^2	6797.450	224.307	0.0001
	Pr > χ^2	< 0.0001	< 0.0001	0.992
SUG	Value	-6.049	-0.219	-0.018
	SE	0.318	0.077	0.166
	Wald χ^2	361.809	8.039	0.012
	Pr > χ^2	< 0.0001	0.005	0.912
GLY	Value	-4.906	-0.768	-0.043
	SE	0.153	0.045	0.083
	Wald χ^2	1024.474	286.648	0.264
	Pr > χ^2	< 0.0001	< 0.0001	0.607
LIP	Value	-4.633	-0.881	-0.086
	SE	0.450	0.141	0.245
	Wald χ^2	106.100	39.178	0.123
	Pr > χ^2	< 0.0001	< 0.0001	0.726

DISCUSSION

In mosquitoes and other insects, life history traits like wing length and pupal weight can be considered indicators of reproductive success and fitness (AGNEW et al. 2000, 2002, BRIEGEL 2003). In different species of mosquitoes the correspondence among pupal weight, wing length, longevity and fecundity justifies the use of one or many life history traits as markers of fitness at the individual or population level (BLACKMORE & LORD 2000, PADMANABHA et al. 2011a, 2011b, MUTURI et al. 2012). The amount of energy reserves in

Fig. 2. The energy reserves (Mean $\pm$ SE) of the mosquitoes expressed as a function of unit pupal weight of the four mosquito species – *Armigeres subalbatus* (Asu), *Culex quinquefasciatus* (Cqu), *Aedes aegypti* (Aae) and *A. albopictus* (Aal) – collected from larval habitats in Kolkata, India. (Gly – glycogen, Sug – sugar, Lip – (lipid)).

an individual mosquito can also be included as an indicator of fitness, owing to the correspondence with multiple life history traits (MOSTOWY & FOSTER 2004, BARGIELOWSKI et al. 2012, MAİGA et al. 2012, KAUFMANN et al. 2013). The link between life history traits and energy reserves form the basis for using them as an indicator of reproductive success and fitness at individual and population levels. Pupal weight and wing length have been incorporated in many entomological surveillance studies to predict population size and abundance and thus the chances of mosquito borne diseases (BLACKMORE & LORD 2000, STRICKMAN & KITAYAPONG 2003, BANERJEE et al. 2013a, 2013b). A positive correlation between pupal weight and energy reserves has been demonstrated in a number of mosquito species, including *Aedes aegypti* and *A. albopictus* (CHAMBERS & KLOWDEN 1990, SHIN et al. 2012). As a result of positive correlations, pupal weight is used as a suitable predictor of adult body size (adult weight and wing length) and energy reserves in mosquitoes. The observations of the present study justify this proposition, since the energy reserves showed correspondence with pupal weight and wing

Fig. 3. The results of the discriminant analysis using pupal weight (PW), wing length (WL), energy reserves (GLY=glycogen, SUG=sugar and LIP=lipid) as explanatory variables against the four mosquito species as response variables (*Aal* = *Aedes albopictus*, *Aae* = *Aedes aegypti*, *Asu* = *Armigeres subalbatus* and *Cqu* = *Culex quinquefasciatus*; ♂ = male and ♀ = female). The results are represented as: (a) a biplot with the ordination of the mosquitoes in two axes, (b) eigenvalues and canonical correlations and the standardized canonical discriminant function coefficients and (c) the Mahalanobis distance matrix for the response variables (values in bold are significant at $P < 0.001$). Wilk's λ yielded a significant F-value justifying the application of discriminant function analysis (Wilk's $\lambda = 0.0002$; $F_{35, 3141} = 624.010$; $P < 0.0001$).

Fig. 4. The degree of sexual dimorphism with reference to energy reserves in four mosquito species. The values of the energy reserves are scaled in accordance with the pupal weight of the individuals to make them comparable among the four species. The values (except *Aedes albopictus* glycogen; $t_{43,(1)} = 2.5$; $P < 0.05$) did not differ significantly from 0 (dashed line, representing no difference) based on the one-tailed t-test.

length of *Aedes aegypti*, *A. albopictus*, *Armigeres subalbatus* and *Culex quinquefasciatus*. Although earlier studies demonstrated the relation between life history traits and energy reserves in *Aedes* mosquitoes (VAIDYANATHAN et al. 2008), the present study is a pioneer effort to report the same for the mosquitoes *Armigeres subalbatus* and *Culex quinquefasciatus* from the geographical region concerned. In most instances, laboratory reared mosquitoes are used for estimating energy reserves, except for the few where field collected individuals are used (STRICKMAN & KITTAAPONG 2003, BANERJEE et al. 2015). Estimation of the pupal weight and energy reserves of field collected mosquitoes collate the amount of resources exploited and assimilated by the larval stages. Variations in energy reserves within and between the mosquitoes thus reflects the acquisition of resources and their subsequent assimilation by the larval stages developing in the respective larval habitats under natural conditions, which was observed in the four mosquito species considered in the present study.

Although mosquitoes may vary in terms of larval developmental time and adult features (LEHMANN et al. 2006, DA-SILVA ARAÚJO et al. 2012), the predictability of pupal weight for the adult body size and energy reserves remains an invariant property. In the present study, the correspondence between pupal weight, wing length and energy reserves substantiates this proposition. However, species-specific variations were observed, with the pupal weight of *Culex quinquefasciatus* and *Armigeres subalbatus* being considerably higher than that of *Aedes aegypti* and *A. albopictus*. The sex specific differences were observed to be an invariant property of the four mosquito species. As a result, the energy reserves per unit pupal weight varied in both a sex-specific and a species-specific manner, represented by the degree of sexual dimorphism. Even though constancy in energy reserves among the species may not be the same owing to differences in feeding activity, resource acquisition and assimilation, the sex-specific differences may remain invariant. The survival and reproductive strategies of the female mosquito differ from the male in many senses, at least one of which is related to egg production and maturation (BRIEGEL 2003). Energy expenditure for egg maturation is somewhat higher than for sperm production, if the unit biomasses of mosquito sperms and eggs are considered. Therefore, females would require a higher amount of energy to mature as an adult than males. However, constancy of biomass and energy reserves in all the species is not observed, owing to the differences in the life history strategies as well as the evolutionary consequences that lead different species of mosquitoes to mature at different sizes and biomass. Thus the observed differences among the species of mosquitoes can be attributed to the way natural selection has shaped them. In general, the energy reserves of the female mosquitoes are higher than their male counterparts as obtained from the laboratory reared populations (FOSTER 1995, 2008, BANERJEE et al. 2015, YU et al. 2016). This may differ when scaled with the pupal weight. The mean biomass of a female pupa was higher than the male one, so unit energy

reserves per pupal biomass may be skewed towards the male. This was observed in the present instance for all the four mosquitoes collected from nature. However, variations in the pupal weight was not considerably high as known for the four mosquito species considered (ADITYA & SAHA 2013, BANERJEE et al. 2013a, 2013b, 2015), thereby leaving a chance of error to generalize the observations of the present study. Energy reserves show a considerable positive correlation with the pupal weight, but may be controlled with the developmental requirements of somatic and reproductive tissues (JULIANO et al. 2004, MURRELL & JULIANO 2008, ARRIVILLAGA & BARRERA 2004, BARRERA et al. 2008). Thus the variations in energy reserves observed in the four co-occurring mosquitoes are a reflection of the differences in their life history strategies and adaptations to environmental conditions. It needs to be tested further whether the variations in energy reserves in these mosquitoes correspond to a preference for oviposition habitat selection and the resources available therein.

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REFERENCES

- ADDIN SOFT SARL 2010. XLSTAT software, Version 10.0. Paris, France.
- ADITYA G., PRAMANIK M.K., SAHA G.K. 2009. Immatures of *Aedes aegypti* in Darjeeling Himalayas – expanding geographical limits in India. *Indian Journal of Medical Research* **129**(4): 455–457.
- ADITYA G., SAHA G.K. 2013. Estimate of survivorship of immature stages of the mosquito *Armigeres subalbatus* (COQUILLET, 1898). *Invertebrate Reproduction and Development* **57**(3): 200–207.
- AGNEW P., HAUSSY C., MICHALAKIS Y. 2000. Effects of density and larval competition on selected life history traits of *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology* **37**(5): 732–735.
- AGNEW P., HIDE M., SIDOBRE C., MICHALAKIS Y. 2002. A minimalist approach to the effects of density dependent competition on insect life history traits. *Ecological Entomology* **27**(4): 396–402.

- ARRIVILLAGA J., BARRERA R. 2004. Food as a limiting factor for *Aedes aegypti* in water-storage containers. *Journal of Vector Ecology* **29**(1): 11–20.
- BANERJEE S., ADITYA G., SAHA G.K. 2013a. Pupal productivity of dengue vectors in Kolkata, India: implications for vector management. *Indian Journal of Medical Research* **137**(3): 549–559.
- BANERJEE S., ADITYA G., SAHA G.K. 2013b. Household disposables as breeding habitats of dengue vectors: linking wastes and public health. *Waste Management* **33**(1): 233–239.
- BANERJEE S., ADITYA G., SAHA G.K. 2015. Household wastes as larval habitats of Dengue vectors: comparison between urban and rural areas of Kolkata, India. *PLoS ONE* **10**(10): e0138082.
- BANERJEE S., ADITYA G., SAHA N., SAHA G.K. 2010. An assessment of macroinvertebrate assemblages in mosquito larval habitats – space and diversity relationship. *Environmental Monitoring and Assessment* **168**(1): 597–611.
- BAQAR S., HAYES C.G., AHMED J. 1980. The effect of larval rearing conditions and adult age on the susceptibility of *Culex tritaeniorhynchus* to infection with West Nile virus. *Mosquito News* **40**: 165–171.
- BARGIELOWSKI I., KAUFMANN C., ALPHEY L., REITER P., KOELLA J. 2012. Flight performance and teneral energy reserves of two genetically-modified and one wild-type strain of the yellow fever mosquito *Aedes aegypti*. *Vector-Borne and Zoonotic Diseases* **12**(1): 1053–1058.
- BARRAUD P.J. 1934. Fauna of British India, including Ceylon and Burma. Diptera (Family Culicidae: Tribes Megarginini and Culicini), vol. V. Taylor and Francis, London, UK.
- BARRERA R., AMADOR M., DIAZ A., SMITH J., MUNOZ-JORDAN J.L., ROSARIO Y. 2008. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Medical and Veterinary Entomology* **22**(1): 62–69.
- BÉDHOMME S., AGNEW P., SIDOBRE C., MICHALAKIS Y. 2005. Pollution by conspecifics as a component of intraspecific competition among *Aedes aegypti* larvae. *Ecological Entomology* **30**(1): 1–7.
- BLACKMORE M.S., LORD C.C. 2000. The relationship between size and fecundity in *Aedes albopictus*. *Journal of Vector Ecology* **25**(2): 212–217.
- BRIEGEL H. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *Journal of Medical Entomology* **27**(5): 839–850.
- BRIEGEL H. 2003. Physiological bases of mosquito ecology. *Journal of Vector Ecology* **28**(1): 1–11.
- BRIEGEL H., TIMMERMAN S.E. 2001. *Aedes albopictus* (Diptera: Culicidae): Physiological Aspects of Development and Reproduction. *Journal of Medical Entomology* **38**(4): 566–571.
- CHAMBERS G.M., KLOWDEN M.J. 1990. Correlation of nutritional reserves with a critical weight for pupation in larval *Aedes aegypti* mosquitoes. *Journal of the American Mosquito Control Association* **6**(3): 394–399.
- CHRISTOPHERS S.R. 1933. The fauna of British India including Ceylon and Burma. Diptera, Family Culicidae, Tribe Anophelini. Vol. IV. Taylor and Francis, London, UK.
- DA-SILVA ARAÚJO M., GIL L.H.S., DE-ALMEIDA E-SILVA A. 2012. Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of *Anopheles darlingi* under laboratory conditions. *Malaria Journal* **11**(261): 1–9.

- FOSTER W.A. 1995. Mosquito sugar feeding and reproductive energetic. *Annual Review of Entomology* **40**: 437–443.
- FOSTER W.A. 2008. Phytochemicals as population sampling lures. *Journal of the American Mosquito Control Association* **24**(1): 138–146.
- GRIMSTAD P.R., WALKER E.D. 1991. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse Virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. *Journal of Medical Entomology* **28**(3): 378–386.
- HURLBERT S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**(2): 187–192.
- JULIANO S.A., LOUNIBOS L.P., O'MEARA G.F. 2004. A field test for competitive effects of *Aedes albopictus* on *Ae. aegypti* in South Florida: differences between sites of coexistence and exclusion? *Oecologia* **139**(4): 583–593.
- KAUFMANN C., COLLINS L.F., BROWN M.R. 2013. Influence of age and nutritional status on flight performance of the Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae). *Insects* **4**(3): 404–412.
- LEHMANN T., DALTON R., KIM E.H., DAHL E., DIABATE A., DABIRE R., DUJARDIN J.P. 2006. Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of *Anopheles gambiae*. *Infection, Genetics and Evolution* **6**(5): 410–416.
- MAÏGA H., DABIRÉ R.K., LEHMANN T., TRIPET F., DIABATÉ A. 2012. Variation in energy reserves and role of body size in the mating system of *Anopheles gambiae*. *Journal of Vector Ecology* **37**(2): 289–297.
- MANLY B.F.J. 1994. *Multivariate statistical methods: a primer*, Second Edition. Chapman and Hall, London, UK.
- MOGI M. 2010. Unusual life history traits of *Aedes (Stegomyia) mosquitoes* (Diptera: Culicidae) inhabiting *Nepenthes* pitchers. *Annals of Entomological Society of America* **103**(4): 618–624.
- MOSTOWY W.M., FOSTER W.A. 2004. Antagonistic effects of energy status on meal size and egg-batch size of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology* **29**(1): 84–93.
- MURRELL E.G., JULIANO S.A. 2008. Detritus type alters the Outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* **45**(3): 375–383.
- MUTURI E.J., BLACKSHEAR Jr. M., MONTGOMERY A. 2012. Temperature and density-dependent effects of larval environment on *Aedes aegypti* competence for an alphavirus. *Journal of Vector Ecology* **37**(1): 154–161.
- MUTURI E.J., KIM C.H., ALTO B.W., BERENBAUM M.R., SCHULER M.A. 2011. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Tropical Medicine and International Health* **16**(8): 955–964.
- NAGPAL B.N., SRIVASTAVA A., SAXEN R., ANSARI M.A., DASH A.P., DAS S.C. 2005. Pictorial identification key for Indian anophelines. Malaria Research Centre (ICMR), New Delhi, India.
- NAKSATHIT A.T., EDMAN J.D., SCOTT T.W. 1999. Partitioning of glycogen, lipid, and sugar in ovaries and body remnants of female *Aedes aegypti* (Diptera: Culicidae) fed human blood. *Journal of Medical Entomology* **36**(1): 18–22.

- NASCI R.S. 1986. The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. *Journal of American Mosquito Control Association* **2**(1): 61–62.
- PADMANABHA H., BOLKER B., LORD C.C., RUBIO C., LOUNIBOS L.P. 2011a. Food availability alters the effects of larval temperature on *Aedes aegypti* growth. *Journal of Medical Entomology* **48**(5): 974–984.
- PADMANABHA H., LORD C.C., LOUNIBOS L.P. 2011b. Temperature induces trade-offs between development and starvation resistance in *Aedes aegypti* (L.) larvae. *Medical and Veterinary Entomology* **25**(4): 445–453.
- PAULSON S.L., HAWLEY W.A. 1991. Effect of body size on the vector competence of field and laboratory population of *Aedes triseriatus* for La Crosse virus. *Journal of American Mosquito Control Association* **7**(2): 170–175.
- RICHARDS S.L., ANDERSON S.L., ALTO B.W. 2012. Vector competence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) for dengue virus in the Florida Keys. *Journal of Medical Entomology* **49**(4): 942–946.
- SHARMILA BHARATHI N., PRASAD NG, SHAKARAD M., JOSHI A. 2004. Correlates of sexual dimorphism for dry weight and development time in five species of *Drosophila*. *Journal of Zoology* **264**(1): 87–95.
- SHIN S.M., AKRAM W., LEE J.J. 2012. Effect of body size on energy reserves in *Culex pipiens pallens* females (Diptera: Culicidae). *Entomological Research* **42**(3): 163–167.
- STRICKMAN D., KITTAYAPONG P. 2003. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *American Journal of Tropical Medicine and Hygiene* **68**(2): 209–217.
- SUMANOCHITRAPON W., STRICKMAN D., SITHIPRASASNA R., KITTAYAPONG P., INNIS B.L. 1998. Effect of size and geographic origin of *Aedes aegypti* on oral infection with dengue-2 virus. *American Journal of Tropical Medicine and Hygiene* **58**(3): 283–286.
- TABACHNICK W.J. 2013. Nature, nurture and evolution of intra-species variation in mosquito arbovirus transmission competence. *International Journal of Environmental Research and Public Health* **10**(1): 249–277.
- TAKAHASHI M. 1976. The effects of environmental and physiological conditions on the pattern of transmission of Japanese encephalitis virus. *Journal of Medical Entomology* **13**(3): 275–284.
- TAKKEN W., KLOWDEN M.J., CHAMBERS G.M. 1998. Effect of body size on host-seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae); the disadvantage of being small. *Journal of Medical Entomology* **35**(5): 639–645.
- TELANG A., FRAME L., BROWN M.R. 2007. Larval feeding duration affects ecdysteroid levels and nutritional reserves regulating pupal commitment in the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *The Journal of Experimental Biology* **210**(5): 854–864.
- TELANG A., LI Y., NORIEGA F.G., BROWN M.R. 2006. Effects of larval nutrition on the endocrinology of mosquito egg development. *The Journal of Experimental Biology* **209**(4): 645–655.
- TSURIM I., SILBERBUSH A., OVADIA O., BLAUSTEIN L., MARGALITH Y. 2013. Inter- and intra-specific density-dependent effects on life history and development strategies of larval mosquitoes. *PLoS ONE* **8**(3): e57875.

- VAIDYANATHAN R., FLEISHER A.E., MINNICK S.L., SIMMONS K.A., SCOTT T.W. 2008. Nutritional stress affects mosquito survival and vector competence for West Nile virus. *Vector-Borne and Zoonotic Diseases* **8**(6): 727–732.
- VAN HANDEL E. 1965. Microseparation of glycogen, sugars, and lipids. *Analytical Biochemistry* **11**(2): 266–271.
- VAN HANDEL E. 1985a. Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association* **1**(3): 299–301.
- VAN HANDEL E. 1985b. Rapid determination of total lipids in mosquito. *Journal of the American Mosquito Control Association* **1**(3): 302–304.
- WHO [WORLD HEALTH ORGANISATION] 2003. Malaria entomology and vector control. Learner's guide. World Health Organization, Geneva.
- YU B-T., DINGA Y-M., MO X-C., LIUB N., LI H-J., MO J-C. 2016. Survivorship and fecundity of *Culex pipiens pallens* feeding on flowering plants and seed pods with differential preferences. *Acta Tropica* **155**: 51–57.
- ZAR J.H. 1999. Biostatistical analysis. Fourth Edition. Pearson Education Singapore Private Limited (Indian Branch), New Delhi, India.

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