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Full Length Research

Influence of Length of Proboscis on the Feeding Rate in Skipper Butterflies (Family: Hesperiidae) from Mayureshwar Wildlife Sanctuary Supe Baramati (India)

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There is a significant interaction among the plant species and butterfly species. Their interaction is leading to develop favourable characters in plants as well as in the butterflies. Hesperiidae butterflies are well recognized for the significantly longer length of their proboscis of mouth parts. In the process of evolution, butterflies gained the advantage of longer proboscis. Due to presence of longer proboscis, the butterflies of family: Hesperiidae are able to visit the host plant flowers with longer tube of corolla. The present deals with study of the Hesperiidae butterflies of Mayureshwar Wildlife Sanctuary, Supe Baramati (India) with reference to size of the body; length of proboscis (mm); diameter of food canal and rate of intake of nectar from the host plant flower (nL/s). The longest proboscis among the Hesperiidae butterflies of Mayureshwar Wildlife Sanctuary, Supe Baramati (India) in present attempt belong to grass-skipper butterfly, *Perichares lotus* (A. Butler, 1870) (Hesperiinae Clade: 113), measuring about 63.486 (± 9.883) units. The shortest proboscis among the Hesperiidae butterflies of Mayureshwar Wildlife Sanctuary, Supe Baramati (India) in present attempt belong to dicot skipper butterfly, *Typhedanus undulates* (Hewitson, 1867) (Eudaminae), measuring about 12.93 (± 1.493) units. With reference to rate of intake of fluid food material (nL/S), dicot skipper butterfly, *Bungalotis quadratum quadratum* (Sepp, 1845) (Eudaminae) reported the highest readings 1282.09 (± 277.14) units.

Keywords: Grass-skipper; Typhedanus; Perichares; Bungalotis; Calpodini.

INTRODUCTION

The most ubiquitous food material favoured by animals like birds, butterflies and bats is the nectar (Nicolson, 2007). Different types of traits in the organisms are supposed to the products of evolution. The traits, especially the behavioural are the specializations for adaptations. The physiological and morphological traits are reflecting on the behavioural traits. The mouth parts in butterflies are the specializations as adaptations for the purpose to collect the nectar from flowers from the available flora (Pellmyr, 2002, Muchhala and Thomson, 2009, Johnson and Anderson, 2010, Karolyi et al., 2012, 2013). According to Kernn, et al. (2005), the mouthparts in butterflies are the conspicuous elongations of the appendages around pre-oral cavity. The proboscis is nothing but, the anterior extension of the part (or parts) around the mouth. Quality in terms of length and diameter of food channel of proboscis get reflect on the behavioural pattern, especially for collection of nectar from host plant flowers. The insects with significantly long proboscis include euglossine bees; some tabanid flies; nemestrinid flies and some hawk moths. In these insects, the proboscis measures twice the body length (Amsel, 1938, Borrell, 2005, Borrell and Krenn, 2006, Pauw et al. 2009, Karolyi et al. 2012, 2014). The insects with significantly long proboscis deserve the advantage to gain access to host plant flowers with long corolla tube. Such significant long proboscis is rare in butterflies. According to Paulus and Krenn (1996), the length of proboscis of the butterflies of most of European species is medium. It measures about two-thirds of the body length. Neotropical Eurybia butterflies (Riodinidae) and for some Neotropical skipper butterflies (Hesperiidae) have recorded significantly longer proboscis. Earlier researchers (Kunte, 2007, Bauder et al. 2011, 2013, 2014) have recorded the proboscis length, in butterflies (belong to Riodinidae and Hesperiidae) exceeding twice the length of body. The butterflies visiting the host plant flowers with long as well as short corolla tube have scarcity in their significantly longer proboscis. According to Agosta and Janzen (2005), the butterflies with scarcity of long proboscis possibly taking a competitive advantage over short-proboscis butterflies.

Role of mouth parts in the rate of feeding is well established fact in animals (Huming birds; Bumble bees and some flies) using nectar as food material On the tongue of a hummingbird: Its role in the rate and energetics of feeding (Hainsworth, 1973; Hainsworth and Wolf, 1976; Inouye, 1980; Harder, 1983; Kunte, 2007; Bauder et al., 2011; Karolyi et al., 2013). Earlier attempts of Kunte, K. (2007) reported that the duration of handling the flower by the butterflies with long proboscis was significantly longer. The efficiency of harvesting the nectar from the flower per unit time is significant and functional constraint for evolving extraordinarily long proboscis. According to Karoyi et al (2013), the process of handling the flower by the butterflies can be divided into the steps like, entering into the flower; taking up the nectar and leaving the flower. Each of the step listed deserve significant efficiency. Butterflies with longer proboscis have to manipulate the uncoiling proboscis spiral and finding an entrance into the flower as well as withdrawing and re-coiling the proboscis. The rate of intake of nectar per unit time determine the efficiency of feeding the butterflies. There is possibility of increase in the duration of handling the flower by butterflies with longer proboscis. The possible reasons for this include problems with flower manipulation, deceleration of nectar intake or a combination of both (Heinrich, 1975; Whitham, 1977; May, 1988).

According to Wolf, et al. (1972), the rate of intake of energy in the form of food material by the animal is directly influencing on the foraging efficiency. Intake rate during feeding influences foraging efficiency. According to Hainsworth et al. (1991). Reproductive fitness and rapid feeding should therefore be favoured by natural selection. Daniel et al. (1989); Kim et al. (2011) and Lee et al. (2014) correlate the nectar feeding through a tubular proboscis with the subject to physical laws of fluid dynamics. The morphological configuration of the feeding apparatus and nectar viscosity modify the rate of nectar intake. Based on the Biophysics Principles, there are several parameters affecting the speed of fluid feeding. Therefore, principles in biophysics help to understand the constraints regarding the evolution of extremely long proboscis in butterflies (Kingsolver and Daniel, 1979, 1995; Lee et al., 2014). Kingsolver and Daniel (1979, 1995) explained the nectar intake rate by the butterflies through the physical law of Hagen-Poiseuille. Accordingly, the nectar intake rate of butterflies should increase linearly with increasing pressure difference produced by a suction pump and increase with the radius of the food canal to the exponent four.

Theoretically, it is expected to decline linearly with escalating proboscis length (Kingsolver and Daniel, 1979, 1995). According to Borrell (2007), the butterflies must compensate for the negative influence of a long proboscis through changes in the radius of the food canal or the size of the suction pump, or otherwise bear this cost through a decreased intake rate (Borrell, 2007).

There are some studies on a few butterfly species and other animals such as euglossine bees, hummingbirds and honeyeaters (Hainsworth, 1973; Kingsolver and Daniel, 1983; May, 1985; Mitchell and Paton, 1990; Molleman et al., 2005; Borrell, 2007). There are rare reports on exact measurements of nectar intake rates combined with morphological quantitative data over a variety of butterfly species. Video recordings of foraging activity of Hesperiidae in the wild and during standardized feeding experiments help explain whether prolonged flower handling times of long-proboscis butterflies result from decelerated nectar intake rates, prolonged flower manipulation times or both. If both of these behavioural aspects were independent of proboscis length, prolonged flower handling times of long-proboscis butterflies would simply result from taking larger amounts of nectar than short proboscis butterflies. The present study presents an integrative approach combining data obtained from behavioural observations and morphological analyses of Hesperiidae butterflies of Mayureshwar Wildlife Sanctuary, Super Baramati India.

MATERIALS AND METHODS

(A). Area of Study and Sampling of the Species:

The study was carried out through the use of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary. This “Mayureshwar Wildlife Sanctuary” belong to Deccan Plateau. It is a part of Supe village (Tal. Baramati Dist. Pune Maharashtra India) (Co-ordinates: 18^0 20' 6" N 74^0 22' 15" E) (Figures 1, 2, 3 and 4). Sampling of butterflies of Family: Hesperiidae was carried out in September, October, 2018 and January, February, 2019. The attempts on the morphometry and feeding by butterflies were carried out through the use of three hundred thirty specimens. All the butterflies were belonging to family: Hesperiidae. Total number of species and genera of Hesperiidae butterflies in the attempt was thirty-four and twenty-one respectively. For each parameter, fifty percent females and fifty percent male specimens of each species were utilized. The hand net was used for the purpose to collect the butterflies.

The feeding trials were carried out for each species of Hesperiidae butterfly. After, the feeding trials, the butterflies were stored in seventy percent ethanol. The phylogeny explained by Warren, et al. (2009) was followed for classification of taxa for collected butterflies.

(B) Measurements of body length; Proboscis length and diameter of food canal:

For the purpose of estimation of rate of intake of nectar by individual butterfly, it is necessary to know the length of proboscis and cross-sectional area of the food canal. Both the parameters of were measured through the method explained by Bauder, et al. (2015). According to Bauder, et al. (2013) and Karolyi, et al (2013), measuring the exact size of suction pumps requires time-costly morphological reconstructions. In the present attempt, it was not manageable for a large sample size. Therefore, the present attempt preferred measuring the body size as a correlate for the size of the suction pump. The body size is known to scale with suction pump size (Karolyi et al., 2013). The ethanol preserved butterfly specimen was drained out completely and processed for pinning. The body of butterfly specimen was pinned in a lateral position to a foam mat. Micrograph of body of butterfly specimen was taken through the use of a Nikon SMZ 1500 stereo-microscope (Nikon, Tokyo, Japan). After taking a micrograph of the body, the proboscis of each butterfly specimen was separated from the head at its base. The proboscis was then made uncoiled and fixed on a foam mat. Insect pins were used for fixing the proboscis on foam mat.

Micrographs of the proboscis were taken using a Nikon SMZ 1500 stereomicroscope (Nikon, Tokyo, Japan) equipped with an Optocam-I digital camera (Nikon). Micrographs of body and proboscis thus obtained were imported to Image J (U.S. National Institutes of Health, Bethesda USA). The length of body of individual butterfly and length of proboscis of respective butterfly were measured with the aid of the segmented line tool. After the length measurement, the proboscis was cut off from it’s base, and the two galeae were separated from each other. one of the galea was mounted onto a microscope slide. The food canal was kept facing upwards. It was embedded in glycerol and covered with a cover-slip. The height of the food canal was measured using a Nikon Eclipse E800 light microscope (Nikon) equipped with a Nikon Fi2-U3 digital camera (Nikon) and the NIS Elements D software (Nikon). The width of the food canal was calculated as the distance in micrometer between two focal planes situated on the lateral wall of the food canal and on the cuticular spines of the dorsal linkage. The study attempt measured the height and width of the food canal in two proboscis regions per galea, located at 10% (proximal) and 80% (distal) of the total proboscis length. Further, the study attempt estimated the cross-sectional area of the food canal of a proboscis in approximation to an ellipse and calculated the mean cross-sectional area of the proximal and distal food canal for each proboscis from respective butterfly.

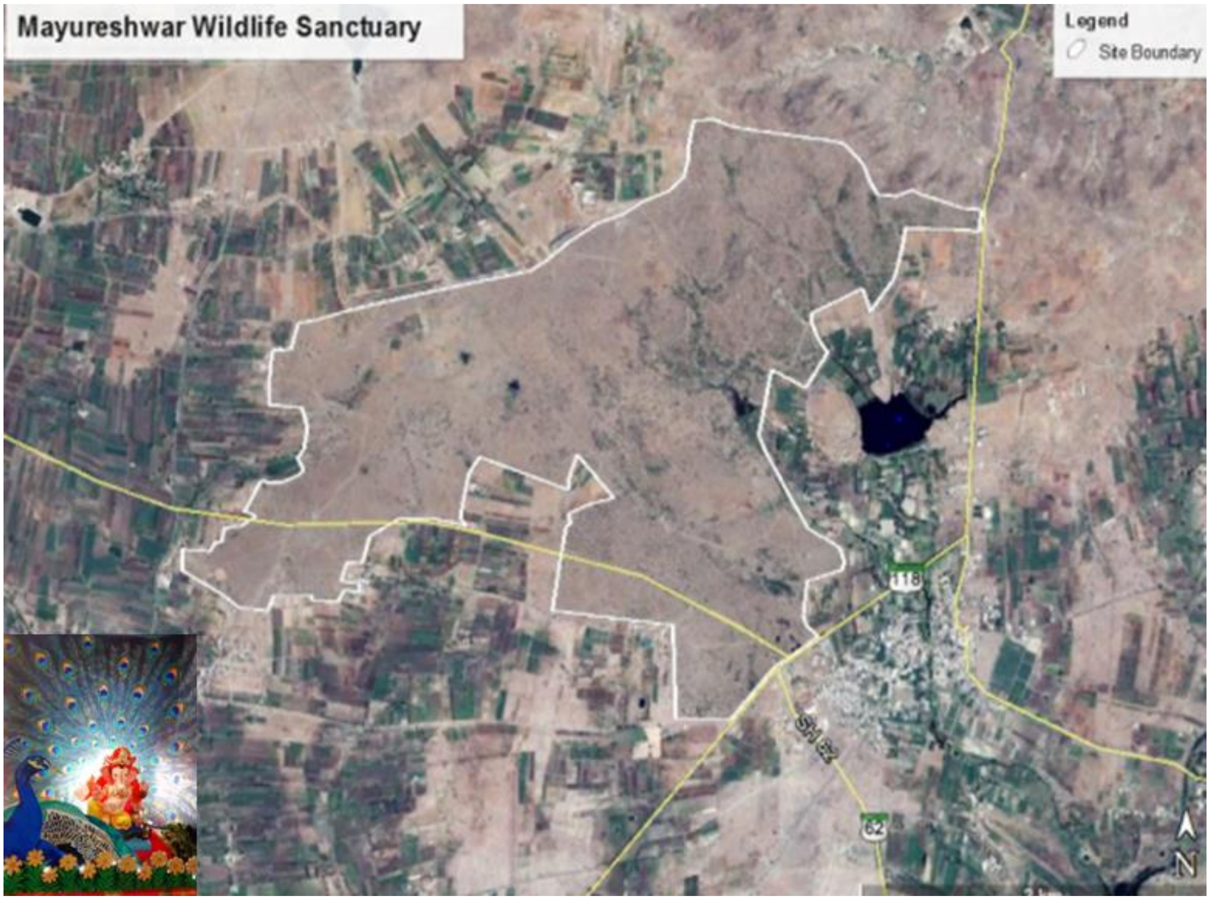

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Figure1. Mayureshwar Wildlife Sanctuary Site.

(C) Attempts on Feeding the Butterflies:

Attempts on feeding the butterflies were carried out in an outdoor cage (39292 m) at the Malegaon Sheti Farm of Agriculture Development Trust, Baramati (India). The hand net collected butterflies from Mayureshwar Wildlife Sanctuary were carried to Malegaon Sheti Farm of Agriculture Development Trust, Baramati (India). Butterflies were stored in a cage until the end of the attempt on feeding. The sampling session for each feeding attempt for each species of butterfly was minimum two hours and maximum four hours. They were firstly made comfortable at the place of working. Butterflies were made free on fresh host plant flowers maintained in the green house at Malegaon Sheti Farm of Agriculture Development Trust, Baramati (India). The captured butterflies were hungry and ready to take the food material. Hungriness of butterflies is obligatory to for subsequent feeding trials. They allowed for taking nectar from flowers of respective host plant. The range of average ambient air temperatures during the attempts on feeding the butterflies was 26^{o}C to 30^{o}C. The sugar solution of forty percent strength was prepared. The contents of this solution include: Sucrose; Glucose; Fructose and distilled water (Alm, et al. , 1990). The artificial nectar was renewed to avoid an increase in concentration due to evaporation. This sugar solution was placed under test conditions for half an hour to warm up to ambient air temperature. Pair of tweezers was used for pinching the wings of butterflies closed. Such individual buttefly was placed on a feeding platform. The glass vial diameter of which was 3.64 mm was kept adjusted to the feeding platform. Glass vial was with markings of levels of fluid. This glass vial was filled with sugar solution. With the help of dissecting needles, the proboscis of individual butterfly was made uncoiled. Such proboscis of individual butterfly was placed onto the sugar solution. The tip of

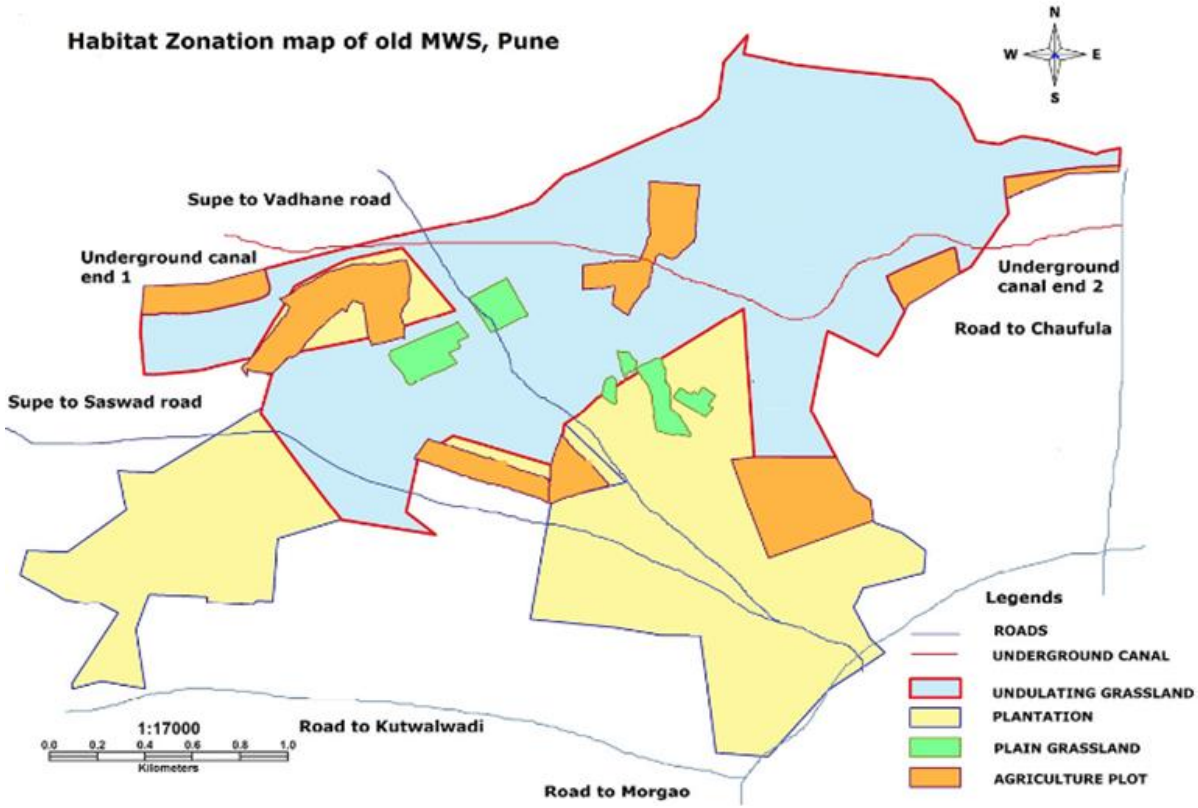

Figure 2. Habitat Zonation of Mayureshwar Wildlife Sanctuary.

proboscis made inserted into the sugar solution. The time of starting and ending of feeding (sucking the sugar solution) was recorded. Thus, individual butterfly was tested once and was subsequently fixed in seventy percent alcohol.

(D) Nectar Intake Rate Assessment:

Through the use of software [PMB 5.0.02.11130 (Sony Corporation)] for continuous video record of feeding, the images were obtained. Images were imported to Adobe Photoshop CS4 Extended 11.0.2 (Adobe Systems Incorporated, San Jose, CA, USA), converted to semitransparency and overlaid. Through the measurements of initial and final levels of fluid in the vial, volume of ingested sugar solution by individual butterfly. The reading of ingested volume of sugar solution and time required were used for calculation of rate of intake of sugar solution. The unit for the rate of intake of sugar solution was nano-Liter per second (nL/S).

(E) Flower Handling Duration Assessment:

The butterflies were collected from the respective host plant flowers from the study area. The butterflies were caught from a flower shortly before they would start taking nectar. The butterflies were then allowed free in an outdoor cage. This cage was equipped with a freshly cut and watered inflorescence of the host plant flower of respective butterflies (the nectar host plant species that butterflies had visited under natural conditions before being collected) (Table 2).

The video recordings were obtained separately for each species of butterfly for visiting host plant flower. Analysis of video recordings of visiting the butterfly to the host plant flower were analysed through the use of software PMB 5.0.02.11130 (Sony Corporation). Assessment of behavioural patterns was carried out through the analysis of video record of the butterfly visiting the host plant flower.

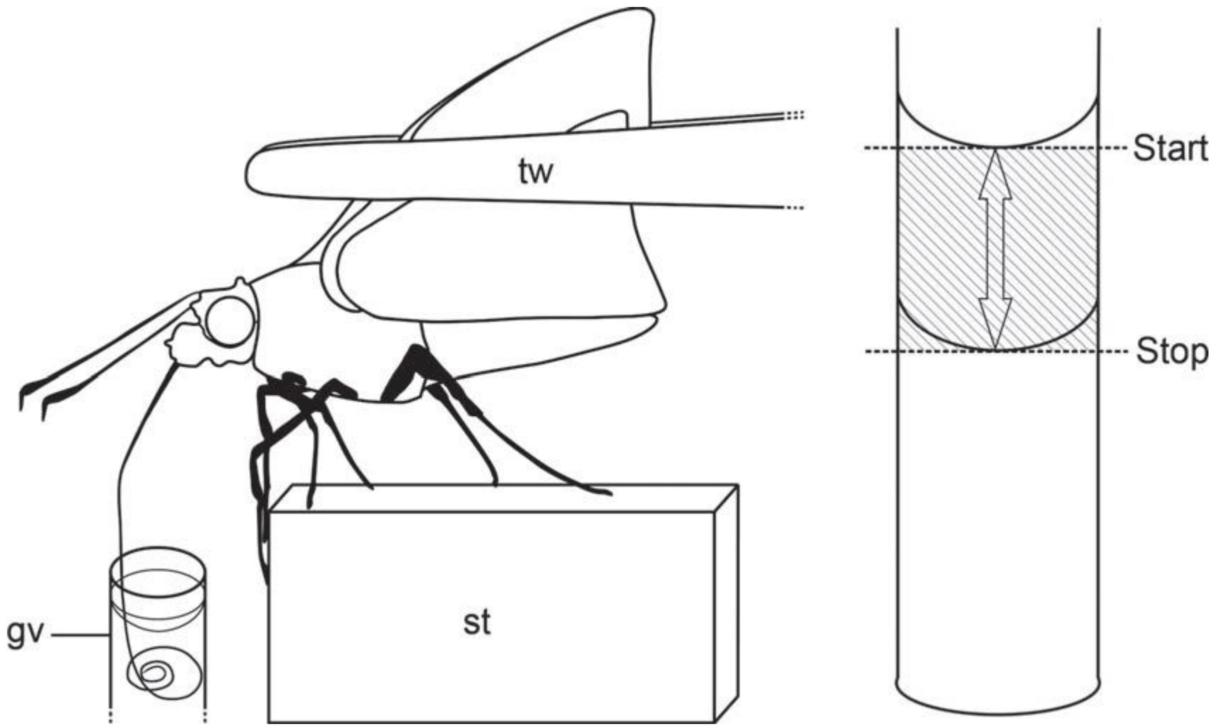

Figure 3. Set-up for video-recorded feeding trials. (a) Skipper feeding from 40% sugar solution. Hungry skippers were locked into position on a stage by pinching the wings together with a pair of tweezers. The proboscis was uncoiled manually and inserted into the glass vial filled with sugar solution. (b) Measuring the ingested volume of sugar solution on video footage. The difference of fluid level from the start and the end of a feeding session was estimated in approximation to a cylinder. gv – glass vial, st – stage, tw – tweezers.

The patterns of behavioural of hesperiidae butterflies in the attempt include: Uncoiling of proboscis; Insertion of proboscis into the corolla tube of host plant flower; proboscis extraction and recoiling of the proboscis after finishing the feeding. The behavioural patterns were assessed as manipulation time. In contrast, the period after successful proboscis insertion when the butterfly remained motionless was evaluated as the duration of suction.

(F). Statistical Analysis of the data:

For consistency in the results, each step in the study attempt was repeated for three times. Statistical package R 3.1.0 (R Development Core Team, 2011) was used for calculation of all the rests. The general linear model (with repeated measurements / random factor: genus) with the function lme of the package nlme (Pinheiro et al., 2014) was used for knowing the influence of sex of butterfly on the rate of intake of nectar fluid.

The influence of sex on rate of intake of nectar was calculated using a general linear model with repeated measurements (random factor: genus) with the function lme of the package nlme (https://cran.r-project.org/web/packages/nlme/nlme.pdf) (Pinheiro et al., 2014). Correlation between variables was assessed using a Pearson correlation with the function of the package Hmisc(Harrell, 2012). The influence of proboscis length and food canal area on intake rate was calculated for a set of 21 genera using the phylogenetic comparative method as implemented in the package ape (Paradis et al., 2004). This method accounts for the phylogenetic relationship between genera as genera cannot be regarded as independent from each other.

RESULTS AND DISCUSSION

The results on the attempt are presented in Tables 1 and 2. The skipper (Hesperiidae) butterflies in the present attempt belong to seven subfamilies: Eudaminae; Pyrginae; Hesperiinae [Clade: 113];

Figure 4. Scanning Electron Micrograph of Proboscis in (Dicot Skipper Butterfly) *Bungalotis quadratum quadratum* (Sepp, 1845) [Family: Hesperiidae; Subfamily: Eudaminae].

Calpodini; Antoptini and Moncini. Number of species of butterflies studied in the attempt was 12; 02 03; 08; 01; 07; and 01 belonging to subfamilies: Eudaminae; Pyrginae; Hesperiinae [Clade: 113]; Hesperiinae [Calpodini]; Hesperiinae [Antoptini]; Hesperiinae [Moncini] and Hesperiinae [Hesperiinae] respectively.

Subfamily - Eudaminae:

The length of the body of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found measured from 16.297 (± 1.443) to 30.625 (± 3.864) units. Minimum body length of the body in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found in *Typhedanus undulates* (Hewitson, 1867) and maximum in *Bungalotis quadratum quadratum* (Sepp, 1845).

The length of the proboscis of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found measured from 13.938 (± 1.829) to 39.691 (± 3.653) units. Minimum length of the proboscis in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found in *Cogia calchas* (Herrich-Schaffer, 1869) and maximum in *Bungalotis quadratum quadratum* (Sepp, 1845).

The food canal diameter (Square Micrometer) in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found measured from 1783.50 (± 9.777) to 9155.781 (± 32.813) units. Minimum food canal diameter in Hesperiidae butterflies from

Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found in *Urbanus simplicius* (Stoll, 1790) and maximum in *Bungalotis quadratum quadratum* (Sepp, 1845).

Rate of intake of food material (in the form of forty percent sugar solution) [nL/S] in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found measured from 78.131 (± 19.817) to 1282.099 (± 277.14) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Eudaminae was found in in *Typhedanus undulates* (Hewitson, 1867) and maximum in *Bungalotis quadratum quadratum* (Sepp, 1845).

Subfamily - Pyrginae:

The length of the body of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found measured from 21.253 (± 1.786) to 23.371 (± 1.963) units. Minimum body length of the body in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found in *Celaenorrhinus darius* (Evans, 1952) and maximum in *Mysoria ambigua* (Mable and Boullet, 1908).

The length of the proboscis of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found measured from 15.412 (± 1.961) to 30.089 (± 3.829) units. Minimum length of the proboscis in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found in *Mysoria ambigua* (Mable and Boullet, 1908) and maximum in *Celaenorrhinus darius* (Evans, 1952).

The food canal diameter (Square Micrometer) in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found measured from 3468.31(± 17.036) to 7323.21 (± 35.971) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found in *Celaenorrhinus darius* (Evans, 1952) and maximum in *Mysoria ambigua* (Mable and Boullet, 1908) .

Rate of intake of food material (in the form of forty percent sugar solution) [nL/S] in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found measured from 137.27 (± 10.413) to 389.83 (± 33.573) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: Pyrginae was found in in *Celaenorrhinus darius* (Evans, 1952) and maximum in *Mysoria ambigua* (Mable and Boullet, 1908) .

Subfamily – Hesperiinae [Clade – 113] :

The length of the body of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found measured from 26.293 (± 1.723) to 29.968 (± 2.516) units. Minimum body length of the body in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found in *Pyrrhopygopsis socretes orasus* (H. Druce, 1876) and maximum in *Perichares lotus* (A. Butler, 1870).

The length of the proboscis of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found measured from 34.654 (± 4.394) to 63.486 (± 9.883) units. Minimum length of the proboscis in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found in *Pyrrhopygopsis socretes orasus* (H. Druce, 1876) and maximum in *Perichares lotus* (A. Butler, 1870).

The food canal diameter (Square Micrometer) in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found measured from 6422.47 (± 33.546) to 7756.33 (± 38.514) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found in *Perichares adela* (Hewitson, 1867) and maximum in *Perichares lotus* (A. Butler, 1870).

Rate of intake of food material (in the form of forty percent sugar solution) [nL/S] in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found measured from 558.62 (± 2.913) to 584.01 (± 3.145) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found in in *Perichares lotus* (A. Butler, 1870) and maximum in *Pyrrhopygopsis socretes orasus* (H. Druce, 1876).

Subfamily – Hesperiinae [Calpodini] :

The length of the body of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was

Table 1. The parameters affecting the rate of feeding in the skipper butterflies (Family: Hesperiidae) from Mayureshwar Wildlife Sanctuary Supe Baramati (India).

S/N	Hesperiidae Butterfly Name	N	Size of the body (mm)	Length of Proboscis (mm)	Food Canal Diameter (Square Micrometer)	Rate of Intake of Food Material [nL/S]
01.	Eudaminae (Dicot Skipper Butterfly) *Astraptes alardus latia* (Evans, 1952).	3	27.200 (± 2.436)	23.674 (± 3.417)	5984 (± 14.786)	486 (±107.556)
02.	Eudaminae (Dicot Skipper Butterfly) *Astraptes anaphus anetta* (Evans, 1952).	3	23.976 (± 2.583)	20.867 (± 3.124)	4875 (± 14.832)	527.028 (±116.635)
03.	Eudaminae (Dicot Skipper Butterfly) *Autochton longipennis* (Plotz, 1882).	9	17.283 (± 1.323)	16.075 (± 2.583)	3689 (± 13.224)	184.796 (± 41.896)
04.	Eudaminae (Dicot Skipper Butterfly) *Autochton zarex* (Hubner, 1818).	6	18.938 (± 1.772)	17.619 (± 1.621)	3401 (± 12.189)	276.499 (± 62.786)
05.	Eudaminae (Dicot Skipper Butterfly) *Bungalotis quadratum quadratum* (Sepp, 1845)	3	30.625 (± 3.864)	39.691 (± 3.653)	9155.781 (± 32.813)	1282.099 (± 277.14)
06.	Eudaminae (Dicot Skipper Butterfly) *Cogia calchas* (Herrich-Schaffer, 1869).	9	17.566 (± 2.216)	13.938 (± 1.829)	2548.91 (± 11.137)	124.613 (± 30.586)
07.	Eudaminae (Dicot Skipper Butterfly) *Spathilepia clonius* (Cramer, 1775).	6	22.162 (± 1.527)	17.584 (± 2.022)	3777.83 (± 16.517)	243.846 (± 61.851)

Table 1. Continued.

08.	Eudaminae (Dicot Skipper Butterfly) *Typhedanus undulates* (Hewitson, 1867).	3	16.297 (± 1.443)	12.931 (± 1.493)	2778.88 (± 12.145)	78.131 (± 19.817)
09.	Eudaminae (Dicot Skipper Butterfly) *Urbanus procne* (Plotz, 1881).	9	20.047 (± 1.271)	15.906 (± 1.837)	3704.55 (± 16.192)	216.71 (± 13.741)
10.	Eudaminae (Dicot Skipper Butterfly) *Urbanus simplicius* (Stoll, 1790).	24	17.833 (± 1.271)	15.324 (± 1.869)	1784.50 (± 9.777)	169.59 (± 11.753)
11.	Eudaminae (Dicot Skipper Butterfly) *Urbanus tanna* (Evans, 1952).	21	19.752 (± 1.418)	16.012 (± 1.952)	2696.25 (± 14.772)	146.97 (± 10.185)
12.	Eudaminae (Dicot Skipper Butterfly) *Urbanus teleus* (Hubner, 1821).	12	19.745 (± 1.417)	16.413 (± 2.089)	2751.33 (± 15.073)	153.97 (± 11.671)
13.	Pyrginae (Spread-winged skippers) Celaenorrhini *Celaenorrhinus darius* (Evans, 1952).	3	21.253 (± 1.786)	30.089 (± 3.829)	3468.31 (± 17.036)	137.27 (± 10.413)
14.	Pyrginae Pyrrhopygini *Mysoria ambigua* (Mable and Boullet, 1908)	12	23.371 (± 1.963)	15.412 (± 1.961)	7323.21 (± 35.971)	389.83 (± 33.573)

Table 1. Continued.

15.	Hesperiinae Clade: 113 (Grass Skipper Butterfly) *Perichares adela* (Hewitson, 1867).	24	29.423 (± 2.471)	56.436 (± 8.786)	6422.47 (± 33.546)	567.055 (± 1.963)
16.	Hesperiinae Clade: 113 (Grass Skipper Butterfly) *Perichares lotus* (A. Butler, 1870).	3	29.968 (± 2.516)	63.486 (± 9.883)	7756.33 (± 38.514)	558.62 (± 2.913)
17.	Hesperiinae Clade: 113 (Grass Skipper Butterfly) *Pyrrhopygopsis socretes orasus* (H. Druce, 1876)	3	26.293 (± 1.723)	34.654 (± 4.394)	6842.15 (± 33.974)	584.01 (± 3.145)
18.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Calpodes ethlius* (Stoll, 1782)	12	26.518 (± 2.115)	42.875 (± 4.421)	5597.11 (± 27.791)	538.47 (± 2.899)
19.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Saliana esperi esperi* (Evans, 1955)	6	18.536 (± 2.201)	35.461 (± 3.656)	3104.85 (± 320.35)	175.29 (± 1.943)
20.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Saliana longirostris* (Sepp, 1840)	3	26.595 (± 3.881)	43.015 (± 4.578)	6067.43 (± 627.02)	433.17 (± 4.762)

Table 1. Continued.

21.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Saliana salius* (Cramer, 1775)	9	23.472 (± 1.664)	47.548 (± 5.742)	5235.31 (± 696.09)	213.78 (± 53.517)
22.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Saliana severus* (Mable, 1895)	3	31.819 (± 2.978)	55.683 (± 7.543)	9147.92 (± 618.15)	802.99 (± 211.01)
23.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Saliana triangularius* (Kave, 1914)	18	21.861 (± 1.516)	41.606 (± 5.636)	4265.37 (± 288.22)	176.09 (± 47.274)
24.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Talides hispa* (Evans, 1955)	3	26.192 (± 1.564)	45.836 (± 2.774)	8231.33 (± 1183.95)	351.57 (± 35.916)
25.	Hesperiinae Calpodini (Grass Skipper Butterfly) *Thracides phidon* (Cramer, 1779)	3	26.695 (± 2.645)	41.529 (± 2.513)	7869.09 (± 848.88)	478.53 (± 49.889)
26.	Hesperiinae Anthoptini (Grass Skipper Butterfly) *Corticea lysias lysias* (Plotz, 1883)	3	13.881 (± 1.806)	14.286 (± 1.587)	1820.71 (± 196.41)	150.96 (± 17.366)

Table 1. Continued.

27.	Hesperiinae Moncini *Cymaenes alumna* (A. Butler, 1877).	6	14.786 (± 1.824)	17.551 (± 1.948)	1584.90 (± 170.97)	69.139 (± 17.348)
28.	Hesperiinae Moncini *Morys geisa* (Moschler, 1879).	24	15.111 (± 1.734)	21.971 (± 2.076)	2012.36 (± 118.54)	76.287 (± 22.298)
29.	Hesperiinae Moncini *Morys micythus* (Godman, 1990).	6	15.011 (± 1.924)	19.746 (± 1.528)	2327.21 (± 169.35)	118.87 (± 26.058)
30.	Hesperiinae Moncini *Papies phaeomelas* (Hubner, 1831).	30	13.902 (± 2.528)	21.783 (± 1.685)	1887.44 (± 328.37)	71.773 (± 19.667)
31.	Hesperiinae Moncini *Papies phainis* (Godman, 1900).	3	17.251 (± 2.042)	20.398 (± 1.651)	1650.72 (± 323.08)	124.91 (± 33.227)
32.	Hesperiinae Moncini *Papies subcostulata* (Herrich-Schaffer, 1870).	36	22.792 (± 1.982)	32.110 (± 1.762)	2769.01 (± 416.46)	90.663 (± 33.823)
33.	Hesperiinae Moncini *Vehilus stictomenes iludens* (Mable, 1891).	6	15.992 (± 2.478)	16.369 (± 3.221)	1371.21 (± 54.583)	36.515 (± 6.297)

Table 1. Continued.

34.	Hesperiinae Hesperiini *Pompeius pompeius* (Latrelle, 1824).	15	20.651 (± 2.604)	19.014 (± 2.113)	3038.46 (± 1.723)	151.11 (± 33.991)

- Each Figure is the mean of three replications.
- Figures with ± sign in parentheses are the standard deviations.

Table 2. Hesperiidae Butterflies used for "Flower Handling Duration Assessment" in the attempt.

S/N	Host Plant: *Stachytarpheta frantzii*	Host Plant: *Calathea crotalifera*
1.	*Autochton longipennis* (N=12).	*Damas clavus* (N=18).
2.	*Urbanus teleus* (N=9).	*Saliana triangularis* (N=15).
3.	*Morys geisa* (N=6).	-

N: Number of Hesperiidae Butterflies Utilized.

found measured from 18.536 (± 2.201) to 31.819 (± 2.978) units. Minimum body length of the body in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Clade – 113]** was found in *Saliana esperi esperi (*Evans, 1955) and maximum in *Saliana severus* (Mable, 1895).

The length of the proboscis of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found measured from 35.461 (± 3.656) to 55.683 (± 7.543) units. Minimum length of the proboscis in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found in *Saliana esperi esperi (*Evans, 1955) and maximum in *Saliana severus* (Mable, 1895).

The food canal diameter (Square Micrometer) in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found measured from 3104.85 (± 320.35) to 9147.92 (± 618.15) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found in *Saliana esperi esperi* (Evans, 1955) and maximum in *Saliana severus* (Mable, 1895).

Rate of intake of food material (in the form of forty percent sugar solution) [nL/S] in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found measured from 175.29 (± 1.943) to 802.99 (± 211.01) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Calpodini]** was found in *Saliana esperi esperi* (Evans, 1955) and maximum in *Saliana severus* (Mable, 1895).

Subfamily – Hesperiinae [Anthoptini] :

The present attempt is reporting *Corticea lysias lysias* (Plotz, 1883) as only one member of subfamily: **Hesperiinae [Anthoptini].** The readings 3.881 (± 1.806) units; 14.286 (± 1.587) units; 1820.71(± 196.41) units and 150.96 (± 17.366) units belongs to the length of the body; the length of the proboscis; the food canal diameter (Square Micrometer) and rate of intake of food material (in the form of forty percent sugar solution) [nL/S] respectively in *Corticea lysias lysias* (Plotz, 1883) subfamily:

Hesperiinae [Anthoptini].

Subfamily – Hesperiinae [Moncini]:

The length of the body of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found measured from 13.902 (± 2.528) to 22.792 (± 1.982) units. Minimum body length of the body in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini] was** found in *Papies phaeomelas* (Hubner, 1831) and maximum in *Papies subcostulata* (Herrich-Schaffer, 1870)..

The length of the proboscis of Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found measured from 16.369 (± 3.221) to 32.110 (± 1.762) units. Minimum length of the proboscis in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found in *Vehilus stictomenes iludens* (Mable, 1891) and maximum in *Papies subcostulata* (Herrich-Schaffer, 1870).

The food canal diameter (Square Micrometer) in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found measured from 1371.21 (± 54.583) to 2769.01 (± 416.46) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found in *Vehilus stictomenes iludens* (Mable, 1891) and maximum in *Papies subcostulata* (Herrich-Schaffer, 1870).

Rate of intake of food material (in the form of forty percent sugar solution) [nL/S] in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found measured from 36.515 (± 6.297) to 124.91 (± 33.227) units. Minimum food canal diameter in Hesperiidae butterflies from Mayureshwar Wildlife Sanctuary Supe Baramati (India) of subfamily: **Hesperiinae [Moncini]** was found in in *Vehilus stictomenes iludens* (Mable, 1891) and maximum in *Papies subcostulata* (Herrich-Schaffer, 1870).

Subfamily – Hesperiinae [Hesperiini] :

The present attempt is reporting *Pompeius pompeius* (Latrelle, 1824) as only one member of subfamily: **Hesperiinae [Hesperiini].** The readings 20.651 (± 2.604) units; 19.014 (± 2.113) units; 3038.46 (± 196.41) units and 151.11 (± 33.991) units belongs to the length of the body; the length of the proboscis; the food canal diameter (Square Micrometer) and rate of intake of food material (in the form of forty percent sugar solution) [nL/S] respectively in *Pompeius pompeius* (Latrelle, 1824) subfamily: **Hesperiinae [Hesperiini].**

Result analysis of present attempt reporting higher rate of intake of fluid solution as a food material by the Hesperiidae butterflies with longer length of proboscis in comparison with the Hesperiidae butterflies with shorter length of proboscis. The rate of intake of fluid solution as a food material by the Hesperiidae butterflies is also influenced by the area of food canal, the proboscis. Both, the length of proboscis and area of food canal in Hesperiidae butterflies serve as crucial parameters for feeding rate. The long proboscis in the Hesperiidae butterflies of Mayureshwar Wildlife Sanctuary Supe Baramati (India) is closely interlinked with ecological adaptations that enable an efficient nectar uptake. Morphological traits (body size); Anatomical traits (musculature) and physiological traits are also important. These traits are serving for development of efficiency of butterflies, which probably enables the development of a large suction pump to overcome nectar flow resistance.

The evidence for morphological adaptations that allow for efficient nectar intake comes from long-proboscis of Eurybia butterflies (Bauder et al., 2013). These butterflies possess larger dilator muscles of the suction pump in relation to the head capsule volume compared to related short-proboscis metalmark species (Bauder et al., 2013). These muscles account for the occurrence of a pressure drop to transport fluid into the gut (Eberhard and Krenn, 2005).

In addition, Eurybia butterflies were also shown to have relatively large food canals (Bauder et al., 2013). Behavioural analyses of skippers during flower visitation confirmed the results of Kunte (2007) by showing that, the butterflies with long proboscis require a longer time for a flower visit.

Further, long-proboscis Hesperiidae species of butterflies spent more time for drinking nectar from a flower. These findings indicate that Hesperiidae butterflies with longer proboscis take higher nectar volumes from the flower with deep corolla tube in *Calathea crotalifera* than butterflies with shorter proboscides from the flowers of *Stachytarpheta frantzii*. As the corolla tube of *Calathea crotalifera* is deeper than that of *Stachytarpheta frantzii*, the Hesperiidae butterflies that visit Calathea flowers most likely ingest higher amounts of nectar. The flowers with deep corolla tube are known to secrete more nectar than the flowers with shorter corolla tube (Harder, 1985; Harder and Cruzan, 1990). Presence of long proboscis can be regarded as an advantage because it enables butterflies to gain access to highly rewarding flowers. However, the flower manipulation times of butterflies increased with proboscis length. Long manipulation times can lower the energy intake rate by decreasing the proportion of foraging time devoted to actually imbibing nectar (Heinrich, 1983; May, 1985). Therefore, longer manipulation times could constitute functional costs of long proboscis.

The problem may be due to a poor supply of mechano- or chemosensory information, as other long-proboscis butterflies (Riodinidae) are endowed with significantly fewer sensilla on their proboscis than related short proboscis species (Bauder et al., 2011, 2013). Alternatively, longer manipulation times of long-proboscis Hesperiidae butterflies could also be due to differences in flower morphology: long-proboscis Hesperiidae butterflies preferred the flower with long corolla tube of *Calathea crotalifera*. The Hesperiidae butterflies with shorter proboscis visited flowers of *Stachytarpheta frantzii* with shorter corolla tubes.

The bumble bees require more time to learn complex flower designs, such as long corolla tubes with concealed nectar, than simple designs (Laverty, 1994). The individual experience gained by butterflies in successive attempts to forage on a flower can shorten flower manipulation time (Lewis, 1986; Kandori and Ohsaki, 1996; Goulson et al., 1997). It clearly means learning the floral morphology by the butterflies could serve as an adaptive strategy for increasing the efficiency of nectar collection (Kandori and Ohsaki, 1996). Further, long-proboscis butterflies could compensate for long manipulation times by visiting fewer nectar-rich flowers instead of many flowers with less volume of the nectar.

In some Hesperiidae butterflies, for example the calpodines, long proboscis may have proven to be useful adaptations to their particular habitat: the deep forests. These butterflies are known to live in shady, forested habitats (Warren et al., 2009). Furthermore, the long-proboscis Eurybia species of butterflies are also known to use the flowers of these plants not only as a nectar source of the adult butterflies, but also as larval food (Schemske and Horvitz, 1984; DeVries, 1997). Some larval instars of butterflies of family: Hesperiidae feed on the leaves of Calathea species (Schemske and Horvitz, 1984).

The larvae of the long-proboscis Hesperiidae species are reported to feed on several monocotyledons (Janzen and Hallwachs, 2009) that occur in the understory of the forest (Weber et al., 2001), including Marantaceae (Calathea sp., Marantasp., Thaliasp.), Costaceae (Costussp.), Heliconiaceae (Heliconiasp.) and Zingiberaceae (Renealmia sp.) (Janzen and Hallwachs, 2009). The convergent evolution of long proboscides in Neotropical deep-forest butterfly species would provide these butterflies exclusive access to deep-tubed flowers, which occur in their microhabitat and cannot be exploited by the vast majority of other butterflies with shorter proboscides. Gaining the access to large amounts of nectar concealed inside long corolla tubed flowers through a long proboscis could also serve to fulfil high energy demands of some butterflies of family: Hesperiidae.

CONCLUSION

The allometric scaling of proboscis length with body size virtually comes at no costs in terms of decreased nectar intake rates. The highest nectar intake rate (1467 nL/s) is achieved by the only long-proboscis species featuring an almost isometric scaling relationship of proboscis length and body size. The dicot skipper butterfly, *Bungalotis quadratum quadratum* (Sepp, 1845) (Eudaminae N=3) in the present attempt reported the highest rate of intake of sugar fluid. This exceptional result reported by dicot skipper butterfly, *Bungalotis quadratum quadratum* (Sepp, 1845) is definitely due to efficiency acquired through longer proboscis, body size and food canal diameter. The suggests that the scaling relationship of body size and proboscis length may influence the nectar intake rate of a butterfly, which can be maximized in a large specimen featuring an isometric scaling relationship. These findings point to the importance of inter-specific variation in scaling relationships and its effect on the foraging efficiency of a butterfly.

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