Biocatalysis and Agricultural Biotechnology 20 (2019) 101188

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

The relationship between genome size, morphological parameters and diet breadth in insect species

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ARTICLE INFO

Keywords:
Insect species
Genome size
Correlation
Morphology
Host range

ABSTRACT

Genome size estimation is the first step involved in the complete genome sequencing project. Knowledge on genome size is also useful in research work related to genetics, molecular biology, and systematics. Though there are many methods of genome estimation, the Propidium Iodide (PI) based flow cytometry is simple and gives an accurate estimation. In the present investigation, the diploid genome size of four insect species *viz.,* cotton leafhopper, *Amrasca biguttula biguttula* (392.47 Mb) (Cicadellidae: Hemiptera), bean pod borer, *Maruca vitrata* (1489.63 Mb) (Crambidae: Lepidoptera), tomato pinworm, *Tuta absoluta* (1128.61 Mb) (Gelechiidae: Lepidoptera) and eggplant shoot and fruit borer, *Leucinodes orbonalis* (944.52 Mb) (Crambidae: Lepidoptera) were estimated using chicken RBC (2.33 pg) as a reference standard. The relationship of genome size with the insect morphological traits and host plant range showed no evident correlation between them.

1. Introduction

An organism cell nucleus being a core element that carries a hereditary blueprint called genetic material. The obvious essentiality of genetic material is the role in cell cycle duration, cell size, and adaptivity under different selection pressure and so on (Leitch and Bennett, 2007). These make the genome size information, a critical for many fields of research that deals with taxonomy, evolutionary changes (Kron et al., 2007) and genome sequencing projects (Rabinowicz and Bennetzen, 2006). The C-value or genome size refers to the amount of DNA present in a cell which is constant for each organism and varies across eukaryotes over 64,000-fold (Pellicer et al., 2018). Eukaryotic genomes not only contain genetic information but also act as structural components that determine nuclear properties and influence various biological features such as cell size, developmental rate, and developmental complexity (Gregory and Hebert, 1999; Koshikawa et al., 2008). Genome size is described by either mass (pg) or the number of base pairs (bp) (Gregory, 2005). So far, the genome sizes of 8005 animal species have been recorded in the animal genome size database (Accessed 15 December 2018) (Gregory, 2018). Compared to those of mammals and birds, the genome sizes of invertebrates remain poorly studied. Of the nearly 1000000 described insect species, the genome sizes of 1345 (0.134%) insects are known with the coverage of species under Lepidoptera and Hemiptera are only around 6.5% each (Gregory, 2018).

Flow cytometry is a convenient and reliable method that has been used to estimate nuclear genome size in many organisms, including plants, animals, and insects (Waring, 1965; Bennett et al., 2003). The variation in genome size in extant species is on the level of ten to hundred thousand folds (Gregory, 2018). DNA flow cytometry explains the use of flow cytometry for the estimation of DNA quantity in cell nucleus. Flow cytometry analyses microscopic particles in suspension that are compelled to move a single flow file within a fluid stream through the focus of intense light. Scattered light pulses and fluorescence are collected and converted to electric pulses by optical sensors and classified.

The cotton leafhopper, *Amrasca biguttula biguttula* (Cicadellidae: Hemiptera), eggplant shoot and fruit borer, *Leucinodes orbonalis* (Crambidae: Lepidoptera), bean borer, *Maruca vitrata* (Crambidae: Lepidoptera) are all originated from India but invaded many other countries across the globe. Whereas the tomato pinworm, *Tuta absoluta* (Gelichiidae: Lepidoptera) have originated from Peru in South America and invaded many other countries including India. All these insects cause severe yield losses in cotton, eggplant, legumes, and tomato

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https://doi.org/10.1016/j.bcab.2019.101188
Received 20 March 2019; Received in revised form 28 May 2019; Accepted 30 May 2019
Available online 31 May 2019
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respectively not only in their country of origin but in all the invaded countries so far (Tonnang et al., 2015).

One of the major rationales for genome size estimation of these insect species is due to their economic importance as dreaded pests of crops and for further work on their genome as well as transcriptome sequencing. Using flow cytometry, it is possible to create a precise and accurate estimate of the diploid genome size of an insect that is useful for genomics, genetics, molecular or cell biology and systematics. Genome size estimation is a basic component in complete genome sequencing and also approximate estimators of the cost and difficulty of genome sequencing programs for the non-model organisms. Bennett et al. (2000) said that the knowledge on the prior estimation of genome size is also essential for gene cloning.

In this study, the diploid genome size of the four economically important insect species was estimated by Propidium Iodide (PI)-based flow cytometry. To our knowledge, we present the first data set that relates the diploid genome size of all the four insect pests and their relation with morphological parameters and host plant range.

2. Materials and methods

2.1. Test insects collection and maintenance

The cotton leafhopper, *A. biguttula biguttula*, and tomato pinworm, *T. absoluta* were and maintained on cotton and tomato plants respectively under net-house conditions at the experimental farm of ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. *L. orbonalis* larvae were reared on potato tuber-based artificial diet. The larvae of *M. vitrata* were reared on soybean-cowpea-wheat-germ based diet developed by Wang et al. (2013) with little modifications. The insect obtained from isogenic populations were used in flow cytometry analysis.

The moths of *T. absoluta*, *L. orbonalis*, *M. vitrata*, and the leafhopper, *A. biguttula biguttula* derived from single isogenic line were released (1:1 sex ratio) in separate oviposition cages provided with fresh host plants/paper towel for mating and egg laying. The cotton wads soaked in sugar solution (0.2%) and honey (0.5%) served as food for the moths. The plant parts/paper towel containing the freshly laid eggs were removed daily and kept for hatching. Newly hatched larvae or the nymphs (leafhopper) were transferred to specimen jars with respective host plants diet. Rearing was continued until the emergence of the adults. The information on the host range of the test insects was obtained from various literature sources.

2.2. Morphometric measurements

The body measurements of the insect species were made for all the stages using stereoscopic microscope (Carl-ZEISS (Zoom 1:10), Model: Discovery. V8, Camera: Axiocam.105 colour) supported with ZEN software. Antennal length, eye width, length and breadth of 20 adults were measured. Correlation between the morphological parameters, number of host plants and the mean genome size were estimated with the Spearman rank correlation using the program SPSS 16.0 (IBM Corp, 2016).

2.3. Preparation of samples for flow cytometry

Intact nuclei suspension for each insect species was prepared by complete homogenization of dissected head samples in 400 µl of Galbraith buffer (45 mM $MgCl_2$; 30 mM sodium citrate; 20 mM MOPS; 0.1% (w/v) Triton X 100; pH 7.0) (Galbraith et al., 1983). The homogenate was passed through a 20µ nylon filter to obtain the cells free from debris. The final volume of the suspension was made to 1.5 ml using the Galbraith buffer. For further enrichment of the nuclei population, cells were centrifuged at 1500 rpm for 2 min and the pelleted cells were resuspended in 1.5 ml of hypotonic solution (Distilled water containing 0.1% trisodium citrate, 40 µg/ml RNAse, 25 µg/ml propidium iodide and 0.03% NP40/igepal) for 15 min.

2.4. Flow cytometry analysis

The samples were subjected to flow cytometry analysis (BD FACS™, USA) (Li et al., 2015) for estimating the genome size. The measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale and 5000–20000 nuclei were analysed for each sample (Galbraith et al., 1983). Nuclei from chicken (*Gallus domesticus*) red blood cells (2.3 pg/2C) were used as an internal reference standard. The estimates of three replicate samples of each insect species were subjected to statistical analysis using simple mean variance analysis in SPSS 16.0 (IBM Corp, 2016).

2.5. Data analysis

Results were obtained in the form of a histogram of relative fluorescence correlating the relative DNA content after comparison with nuclei of a reference standard, whose genome size is known (Galbraith et al., 1983). The absolute DNA content of a sample was calculated on the values of the G_1 peak means

$$\text{Sample 2C DNA content} = \frac{(\text{sample G1 peak mean})}{(\text{standard G1 peak mean})} \times \text{standard 2C DNA content (pg DNA)}$$

3. Results

3.1. Genome size estimation

The genome sizes of four economically important insect species were estimated by flow cytometry. The chicken erythrocyte cells were the reference standard (Fig. 1). All the samples were replicated thrice and showed good reproducibility. The estimated genome sizes of the lepidopteran insects *viz., L. orbonalis, T. absoluta* and *M. vitratta* were 944.52, 1128.61 and 1489.63 Mb respectively. The genome size of hemipteran insect, *A. biguttula biguttula* was estimated at 392.47 Mb (Table 1). The fluorescence histograms of genome size assessments were depicted in the form of G1 peak mean (Figs. 1–3). These data indicate that the lepidopteran insects have relatively bigger genome size than the hemipteran bug.

Table 1
Genome size estimates of economically important insect species.

Insect species	Family/Order	Principal host plant(s)	2C- value (pg)	n	Diploid genome size (Mb)
T. absoluta	Gelechiidae: Lepidoptera	Tomato	1.165919	3	1128.61 ± 2.98
L. orbonalis	Crambidae: Lepidoptera	Brinjal	0.975744	3	944.52 ± 10.07
A. biguttula biguttula	Cicadellidae: Hemiptera	Cotton, ladies finger	0.405444	3	392.47 ± 3.11
M. vitrata	Crambidae: Lepidoptera	Cowpea, lima bean, common bean, pigeon pea	1.538874	3	1489.63 ± 5.64

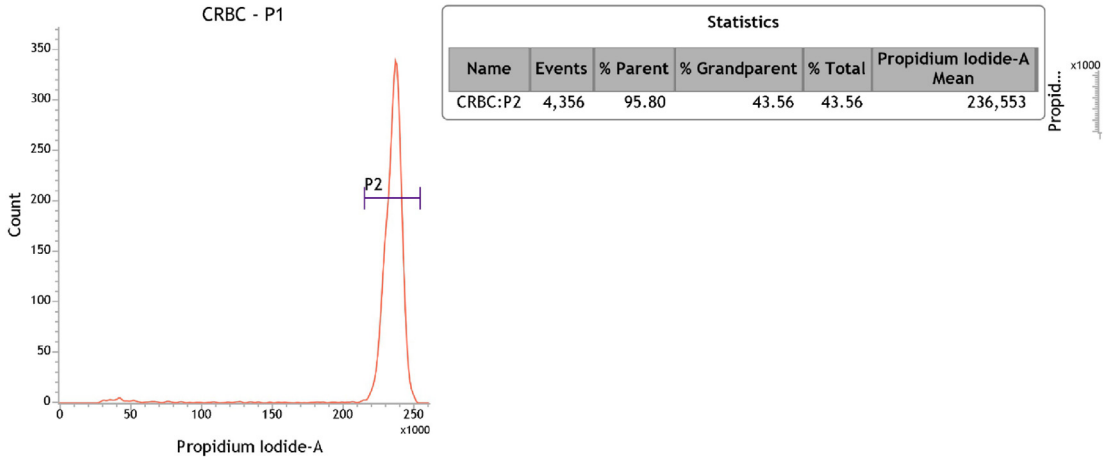

Fig. 1. Flow cytometric fluorescence histogram of reference chicken RBC cells.

3.2. Correlation of genome size with morphology and host range

The morphological data were obtained based on the adult size measurement, which includes eye width (EW), antennal length (AL), body length (BL), and body width (BW) of all insects. Correlation between the insect morphology and genome size depict that, there was no influence of morphology on insect genome size. Because, the small insect like *T. absoluta* has greater genome size compared to *L. orbonalis,* which belong to the same order Lepidoptera with different morphology. Similarly, the diploid genome sizes of all four insects did not correlate with their host plant range *viz.,* 22, 21, 25 and 13 of *T. absoluta*, *L. orbonalis*, *A. biguttula biguttula* and *M. vitrata* (Table 2) respectively (https://www.plantwise.org).

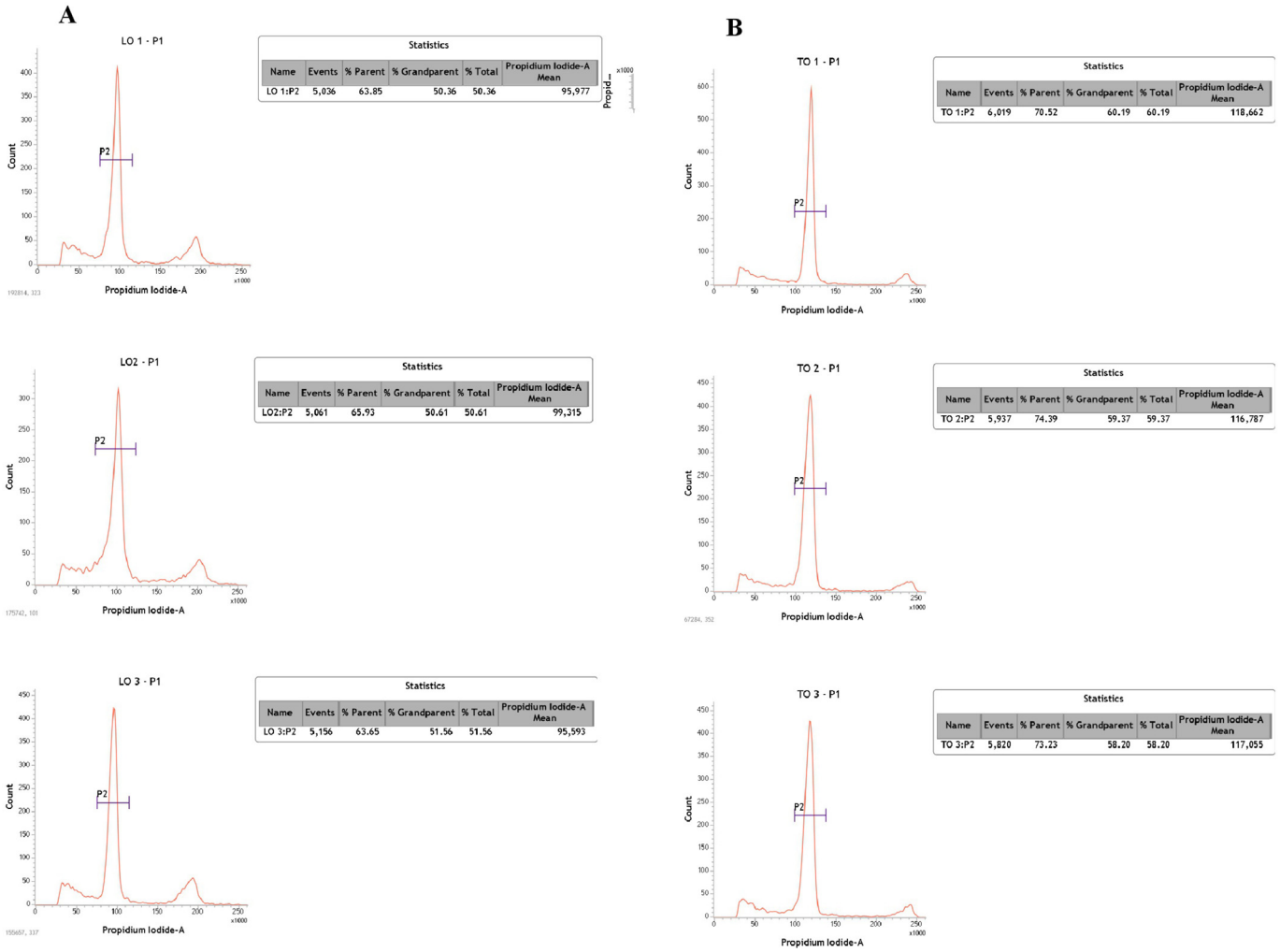

Fig. 2. Flow cytometric fluorescence histograms of *Leucinodes orbonalis* (A) and *Tuta absoluta* (B) cells.

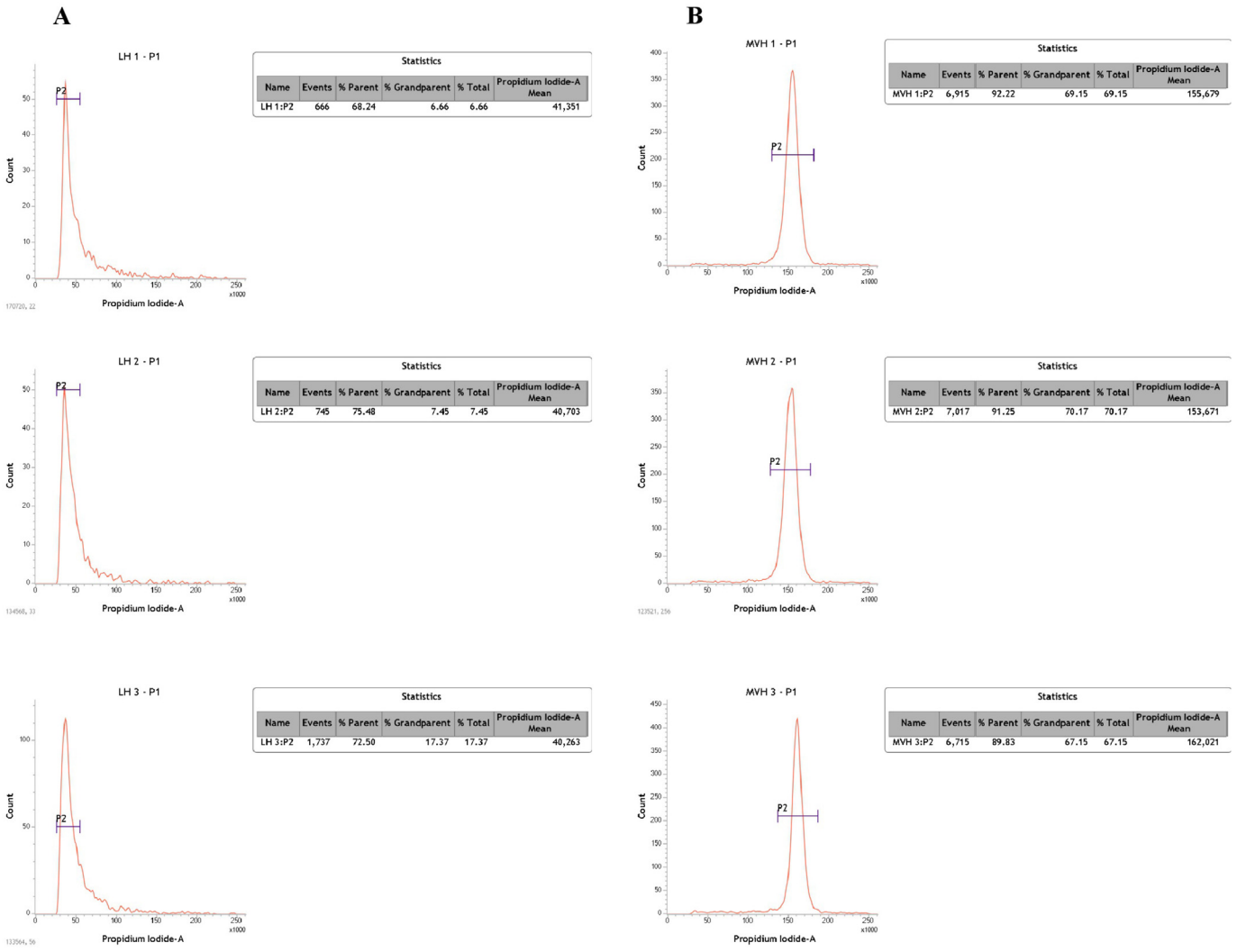

Fig. 3. Flow cytometric fluorescence histograms of *Amrasca biguttula biguttula* (A) and *Maruca vitratta* (B) cells.

4. Discussion

To explore the genome size, we performed C-value measurements for four important insect pest species (three from Lepidoptera and one from Hemiptera) using flow cytometry. Flow cytometry, a mere comparative analysis depends on the selection of the standard references. Galbraith et al. (1983) observed differential staining intensity of PI to a type of DNA. In their observation on the binding capacity of PI among plants and animals, they observed that PI binds more to methylated DNA than unmethylated DNA. PI being a non-base pair specific fluorochrome irrespective of species, its binding saturates in 1hr and the stain remains constant for 1–24 h (Bennett et al., 2003).

Rapid analyses of flow cytometry for estimating the size of haploid genome with known standard facilitated many researchers to predict the genome size of unknown organisms. Loureiro et al. (2007) confirmed the genome size of *B. tabaci* (640–680 Mb) with *D. melanogaster* (176 Mb) as a standard. Similar findings were obtained on other important insects like the honey bee, *Aphis melliferra* (234.7 Mb) (Ardila-Gracia et al., 2010), mosquito *Anopheles gambiae* (264 Mb) (Holt et al., 2002) and *Bombyx mori* (508 Mb) (Rasch, 1974).

The genome size of two different orders *viz.,* Lepidoptera and Hemiptera displayed greater variation in this study. Alfsnes et al. (2017) depicted the genome size varies both within and between taxonomic levels in animals as well as plants. Over the evolution, duplication of the whole genome or accumulation of noncoding elements like transposable and repetitive elements would influence the small or large genome size. The lack of correlation between phylogeny and host plant range is questionable, as it has been reported that phylogenetically related insects are associated with phylogenetically related host plants and this association is ancient (Kergoat et al., 2005, 2012). Similarly, Insecta and Crustacea a weaker association between genome size and phylogeny, suggesting life cycle strategies and habitat as more

Table 2
Correlation analysis of genome size with respect to morphology and host range.

Species	Eye Circumference	Antennal Length	Body Width	Body Length	Host range	R^2
T. absoluta	0.87 ± 0.41 (r = 0.55)	3.84 ± 0.36 (r = −0.55)	1.01 ± 0.04 (r = −0.58)	2.77 ± 0.03 (r = −0.50)	22 ± 0.04 (r = −0.25)	0.36
L. orbonalis	3.84 ± 0.04 (r = 0.26)	18.3 ± 0.08 (r = 0.25)	10.3 ± 0.03 (r = 0.22)	25.52 ± 0.05 (r = −0.25)	21 ± 0.04 (r = −0.52)	0.61
A. biguttula biguttula	1.01 ± 0.36 (r = −0.23)	1.27 ± 0.41 (r = −0.23)	7.5 ± 0.02 (r = −0.23)	3.74 ± 0.04 (r = 0.22)	25 ± 0.21 (r = −0.25)	−0.24
M. vitrata	2.77 ± 0.04 (r = −0.34)	17.43 ± 0.02 (r = 0.45)	10.95 ± 0.21 (r = 0.37)	23.76 ± 0.03 (r = −0.20)	13 ± 0.04 (r = 0.09)	0.51

important determinants (Alfsnes et al., 2017).

Considering all the obvious variations, differences in the DNA references standard and dyeing time explains the probable distortions of genome sizes from their accurate values. Hence, we suggest a closely related species as a standard reference and staining time of PI for 15 min. In our unpublished data on whole-genome sequencing of *L. orbonalis*, we discovered more than 35.4% of the genome is repeat-rich, non-coding DNA sequence. Determining the complete genome sequence of these important pests will fetch informative data. Many factors affects genome size, such as polyploidy, accessory chromosomes, endoduplications (Uozu et al., 1997; John and Miklos, 1988; Ullmann et al., 2005), intron size (Moriyama et al., 1998), presence of microsatellites (Warner and Noor, 2000) and transposons (Vieira et al., 2002).

No correlation between the genome size and the insect's host plant range could be derived in the present study. In contrast, Calatayud et al. (2016) reported that the genome size was influenced not only by host plant range but also by climatic from 21 distantly related lepidopteran insects. On the contrary, a negative correlation was reported between host plant range and genome size in *Helicoverpa* species (Zhang et al., 2019). The evolution of solanaceous specialist *H. assulta* (430 Mb) from the generalists *H. virescens* (408 Mb), *H. armigera* (394 or 337 Mb) and *H. zea* (363 Mb) indicates that the expansion of host plant range is inversely correlated with the genome size in this closely related terminal lineage (Kogan et al., 1978; Fitt, 1989; Mitter et al., 1993; Jallow et al., 2004; Cho et al., 2008; Zhang et al., 2019).

Hence, the main mechanisms contributing to genome size variations are polyploidy and accumulation of DNA sequence repeats (Pellicer et al., 2018) rather than the insects' morphological features or host plant range. The causes of expansion and shrinkage of genome size will require a closer look on their genetic make-up that includes the number and length of repeats comprising transposons and microsatellites and also the size of introns between the coding regions. Further, whole genome sequencing projects would focus on these elements for the reason for genome size variations between and among species to draw a conclusion on genome evolution and parameters that influence their variations.

Conflicts of interest

The authors in this manuscript have no conflict of interest.

Declarations of interest

None.

Acknowledgment

This work was supported by funding from Indian Council of Agricultural Research through CRP on Genomics project. The guidance and timely release of fund from ICAR-NBFGR are greatly acknowledged. K.B. and A.C.G are grateful to financial support received from DST-INSPIRE (2016/IF160900 and 2017/IF170435). Thanks to Prof. Krishnamurthy, National Centre for Biological Sciences for providing flow cytometry facility. The technical suggestion rendered by Dr. Ramkumar G. NPDF, ICAR-IIHR is greatly acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101188.

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