Research Paper

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Comparative Analysis of Codon Usage Bias Between Two Lepidopteran Insect Species: *Bombyx Mandarina* And *Ostrinia Furnacalis*

Biotechnology

KEYWORDS : codon usage bias, ENC, GC, lepidopterans

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ABSTRACT

Background: Codon usage bias is the unequal use of synonymous codons; some codons are more preferred than others. However codon usage pattern is different among genes and among organisms. The bias is found more often in highly expressed genes. The greatest diversity of insects among the animal kingdom makes insects appropriate for codon bias study at variable evolutionary time scales.

Result and Discussion: we analyzed pattern of codon usage in two insect species of Lepidopteran order. The ENC values range from 51 to 60 with a mean value of 57.75 in B. mandarina but from 52 to 60 with a mean value of 57.17 in O. furnacalis, respectively. In B. mandarina, a significant positive correlation was observed between A% and A3% ($r = 0.608^{**}$, $p < 0.01$), *C% and C3 %* ($r = 844^{**}$, $p < 0.01$), *GC% and GC3 %* ($r = 0.818^{**}$, $p < 0.01$) *and significant negative correlation observed between the most of heterogeneous nucleotide comparisons; A% and GC3%* ($r = -0.641^{**}$ $p<0.01$), *T% and G3%* ($r = -0.311$ $p>0.05$), *T % and C3 %* ($r = - 0.871^{**}p<0.01$). *In O. furnacalis , a significant positive correlation was observed between A% and A3 %* ($r = 0.892^{**}p<0.01$), *C% and C3%* ($r = 0.974^{**}$ $p<0.01$), *GC% and GC3%* ($r = 0.895^{**}$ $p<0.01$) *and significant negative correlation observed between the most of heterogeneous nucleotide comparisons; A % and GC3 %* ($r = -0.791^{**}$ $p<0.01$), *T% and G3%*($r = -0.486^{*}$ $p<0.05$), *T% and C3 %*($r = - 0.900^{**}$ $p<0.01$). *These results suggest that compositional constraint under mutation pressure has contributed the pattern of codon usage bias in these two insect species of the order Lepidoptera.*

Conclusion: Codon usage bias was not very remarkable in these species. Nucleotide constraint and compositional constraint are significant factors that affect codon usage pattern in these species. The most frequent codons end with C at the 3rd position, most probably suggesting the role of the compositional constraint under mutation pressure. We find that mutation pressure is the main factor for the pattern of codon usage bias in these Lepidopteran species.

Introduction

In genetic code, most amino acids are encoded by multiple codons, typically differing only at the third position of the codon, i.e. synonymous codons [1]. There are a total of 64 codons, out of these 61 codons code for 20 different amino acids and the remaining three function as stop codons (UAA, UAG, UGA).The frequency of synonymous codon usage is unequal within and among different organisms [2][3][4][5]. Codon usage bias refers to the non-uniform usage of codons.Various factors such as expression level, gene length, composition bias (%G+C content and GC skew), recombination rates and RNA stability, among others, are known to influence the codon bias [6][7][8][9]. In some species, mutational biases or selection for genomic base composition also play a major role in codon usage bias [10]. The nucleotides viz: A, T, C, G of DNA sequence are rarely found in equal proportion in absence of natural selection. The pattern of codon usage bias varies in different species. Different pattern observed in different genes in the same genome based on location is due to mutation bias [11]. The decay of nucleotide bases is due to mutation biases [12]. Moreover, the replication error and DNA repair are not uniform, which cause codon usage bias [13]. The occurrence of A or C in one of the strand in a DNA duplex implies T or G in the other the strand, so the base composition in DNA is described by GC content. The GC content differs from species to species. GC contents among species are wide if the 3rd position of codon is considered. GC contents and mutation bias are correlated [11]. GC bias plays a key role in codon bias in prokaryotes [14] human [15], which results in a close relation between overall GC% and GC% at the 3rd codon position [16].

Phylum Arthropoda shows the largest diversity in animal kingdom. Lepidoptera is the second largest order in the class Insecta that includes butterflies and moths (both called lepidopterans) . Nearly all lepidopteran larvae are called caterpillars. Although many lepidopterans are valued for their beauty, and a few are useful in commerce (e.g., the silkworm, *Bombyx mori*), the larvae of these insects are probably more destructive to agricultural crops and forest trees than any other group of insects. The Asian corn borer, *Ostrinia furnacalis*, is a major pest of corn (maize) in eastern and south-eastern Asia. Five species of the *O. furnacalis*species complex (*O. furnacalis*, *O. orientalis*, *O. scapulalis*, *O. zealis* and *O. zaguliaevi*) occur in japan[17].

The present work has been undertaken to understand the patterns of codon usage bias among the two Lepidoteran insect species: *Bombyx mandarina* and *Ostrinia furnacalis* . Although codon usage bias studies are found within the genus *Drosophila* or between a few related insect species [18][19][20], complete analysis of codon usage bias between the coding sequences of these two insect species is missing. So, the aim of this study is to perform a comparative analysis of codon usage pattern between these two insect species: *Bombyx mandarina* and *Ostrinia furnacalis*. In these two species we analyze a few selected genes which have an exact multiple of three bases. Our results provide useful insights on the patterns of codon usage bias that might help in better understanding of evolution of the genes in insects.

Materials and methods

Sequences data:

The complete coding sequences (cds) of selected genes for two insect species were downloaded from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/Genbank/). GenBank database.

Compositional properties:

Overall nucleotide composition (A%, C%, T% and G %) and nucleotide composition at the wobble position of each codon (A3 %, C3 %, T3 % and G3 %) were analyzed for the insect CDSs using an in-house PERL script written by Supriyo Chakraborty. The GC and GC3 index referred to the overall G + C content and the G+C contents at the wobble position(3rd) of synonymous codons.

Relative Synonymous Codon Usage (RSCU):

Relative synonymous codon usage (RSCU) is a measure of codon bias. RSCU value of a codon greater than 1.0 indicates that the codon is more frequently used than expected whereas the reverse is true for RSCU values less than 1.0 [21]. RSCU value calculated as:

$$RSCUij = \frac{Xij}{\frac{1}{ni}\sum_{j=1}^{ni} Xij}$$

Where, X_{ij} is the frequency of occurrence of the j^{th} codon for i^{th} amino acid (any X_{ij} with a value of zero is arbitrarily assigned a value of 0.5) and n_i is the number of codons for the i^{th} amino acid (i^{th} codon family).

The Effective Number of Codons (ENC):
The effective number of codons used by a gene (ENC) is calculated using the following formula [22]. The values of ENC range from 20 (when only one codon is used for each amino acid) to 61 (when all the codons are used randomly).

$$ENC = 2 + \frac{9}{F_2} + \frac{1}{F_3} + \frac{5}{F_4} + \frac{3}{F_6}$$

Codon Adaptation Index (CAI):
The codon adaptation index (CAI)[28] is an effective measure of codon bias in prokaryotes[23][24][25] and eukaryotes [26][27][28]. CAI is a measurement of the relative adaptedness of the codon usage of a gene towards the codon usage of more expressed genes. The relative adaptiveness (ωk) of each codon is the usage of each codon divided by the most abundant codon within the same synonymous family. CAI values range from 0 to 1, with higher values towards 1 indicating a higher proportion of the most abundant codons [29]. The CAI is calculated as

$$CAI = \exp\left(\frac{1}{L}\sum_{k=1}^{L} \ln \omega k\right)$$

Where ωk is the relative adaptiveness of the kth codon and L is the number of synonymous codons in the gene.

The parameters of codon usage bias for the cds were estimated using an in-house PERL programme developed by Supriyo chakraborty .

Statistical analysis:
Correlation analysis was done to identify the relationship between general nucleotide composition and each base in the wobble codon position, ENC with GC . All the statistical analyses were done using the SPSS software.

Results:

Compositional properties

The genes of *Bombyx mandarina* and *Ostrinia furnacalis* with their accession no, gene length, ENC, CAI and overall GC(%), GC1(%), GC2(%) and GC3(%) are shown in Table 1 and 2(Supplementary material). The overall nucleotide composition and nucleotide composition at the third codon position of *Bombyx mandarina* and *Ostrinia furnacalis* coding sequences are provided in Table 3 and 4 (supplementary material). In *B. mandarina* species, A and G occurred more frequently than C and T and *O.furnacalis* A and T occurred more frequently than G and C as shown in Fig. 1. C occurred most frequently at the third codon position (average C3 % = 29.466 ± 10.30; 30.15 ± 8.25) and A occurred least frequently (average A3 % =21.90 ± 5.82 ; 20.61 ± 5.99) as shown in Fig. 1 for *B. mandarina* and *O. furnacalis* respectively. The overall nucleotide composition and the composition at the third codon position in *B. mandarina* and *O. furnacalis* genes suggests that compositional constraint might be influencing the codon usage pattern of these species. The overall GC % and GC3 % averages in *B. mandarina* are 47.94 ± 5.99 and 52.63 ± 11.41 respectively and in *O. furnacalis* are 47.07 ± 6.62 and 57.48 ± 10.35 respectively.

The ENC values of *B. mandarina* and *O. furnacalis* range from 51 to 60 with a mean value of 57.75 and from 52 to 60 with a mean value of 57.17 respectively (Fig. 2). Higher ENC means lower bias. This result indicates that codon usage bias is not remarkable in these species and is apparently maintained at a stable level. CAI is used to measure the level of gene expression. Gene expression is more in *B. mandarina* than *O. furnacalis* but not statistically significant (p>0.05) as shown in Fig. 3.

Codon usage in insect Species:

aThe overall RSCU values for the 59 codons in *Bombyx mandarina* and *Ostrinia furnacalis* indicated that C occurred most frequently at the third codon position (as shown in Table No 5 and 6 supplementary materials). In *B. mandarina* and *O. furnacalis* the most frequently used codons end with C at 3rd position and are same. These codons are TTC, CTC, ATC, TCC, AGC, ACC, GCC, TAC, CAC, AAC, GAC, TGC and GGC. In *O. furnacalis,* some other codons (GTC, CCC, CGC) are frequently used. In both the species codons ending with G at 3rd codon position are same and these are TTG, CTG, GTG, CCG and AAG. In addition, other codons like CAG, AGG occur in *B.mandarina* and codons like GCG, CGG in *O. furnacalis.* Codons ending with T at 3rd position are ACT, GCT in both the species, in addition to other codons like GTT, TCT, CCT, CGT, GGT in *B. mandarina.* Codons ending with A at 3rd position are GGA, AGA, GAA in both the species. In addition, other codons like CAA, ACA,CCA and TCA occur in *O. furnancalis.* These results indicate that the codon usage pattern in these two insect species is mostly contributed by compositional constraints. Fig. 4 shows comparison of RSCU values in *B. mandarina* and *O. furnacalis.*

Amino acids contribute differently to a gene's codon usage bias (CUB)

Except methionine and tryptophan, the amino acid usage is different in the genes as shown in Table 7 and 8 (Supplementary material). Fig. 5 shows that leu is clearly the singleton amino acid that accounts for the greatest usage and cys accounts for the least usage in genes of both *B. mandarina* and *O. furnacalis* respectively.

Effect of mutational bias on codon usage variation

To identify whether the evolution of codon usage bias in *B. mandarina* and *O. furnacalis* had been driven by mutation pressure alone or whether the translational selection had also played a major role, we compared the correlation between general nucleotide composition (A%, T%, G%, C%, GC%) and nucleotide composition at the third codon position (A3%, T3%, G3%, C3 %, GC3%) of *B. mandarina* and *O. furnacalis* using the Spearman's rank correlation analysis method as shown in Table 9 and 10 respectively.

In *B. mandarina*, a significant positive correlation was observed between A% and A3% (r = 0.608**, *p < 0.01*), C% and C3 % (r = 844**, p < 0.01), GC% and GC3 % (r = 0.818**, p < 0.01) and significant negative correlation observed between the most of heterogeneous nucleotide comparisons; A% and GC3% (r = -0.641** p<0.01), T% and G3% (r = -0.311 p>0.05), T % and C3 % (r = - 0.871**p<0.01). In *O. furnacalis* , a significant positive correlation was observed between A% and A3 % (r = 0.892**p<0.01), C% and C3% (r = 0.974** p<0.01), GC% and GC3% (r = 0.895** p<0.01) and significant negative correlation observed between the most of heterogeneous nucleotide comparisons; A % and GC3 % (r = -0.791** p<0.01), T% and G3%(r = -0.486* p<0.05), T% and C3 %(r = - 0.900** p<0.01). These results suggest that compositional constraint under mutation pressure has contributed the pattern of codon usage bias in two insect species within lepidoptera. However a significant positive correlation between T% and T3% (r =0.741** p<0.01), G% and G3% (r =0.575** p<0.01) and a negative correlation between GC% and T3% (r = - 0.657** p<0.01) in *B. mandarina* and similarly, a significant positive correlation between T% and T3% (r =0.874** p<0.01), G% and G3% (r =0.505* p<0.05) and a negative correlation between GC% and T3% (r = - 0.897** p<0.01) in *O. furnacalis* support the hypothesis that mutation pressure has been a major factor for the pattern of codon usage bias in these species. In addition, negative correlation (r=0.263, p>0.05) between ENC and GC in *B.mandarina* (Fig. 6) and significant negative correlation (r=-0.761, p<0.01) between ENC and GC in *O. furnacalis (*Fig.7) further suggest that mutation pressure is responsible for codon usage bias in these insect species.

Discussion

The present investigation highlights the codon usage patterns in a comparative analysis between the two important insect species; *Bombyx mandarina* and *Ostrinia furnacalis* belonging to the order Lepidoptera. Analysis of codon usage bias is a well-established technique for understanding the evolution of the protein coding sequences of genomes. As usage of synonymous codons during translation is non-uniform, identifying the patterns of codon usage bias is important for understanding the mode of translational selection of protein coding genes among related species. The study of codon usage bias is gaining momentum with the initiation of whole genome sequencing of numerous organisms [30]. Molecular evolutionary investigations suggest that codon usage bias varies both within and between genomes and may have a major impact in understanding the genome evolution among related species [31]. The investigation of the causes and consequences of the pattern of codon usage bias and the detection of selective forces that shape their evolution are of practical importance to the studies of genome biology.

Comprehensive analysis of codon bias between various Lepidopteran insect genomes is lacking. The insect species analyzed in the study are important because they are relevant to the transmission of various plant diseases and are currently considered as important agricultural pests for many crop plants. An analysis of the usage of synonymous codons found strong evidence that in highly expressed *Drosophila* genes codon bias is, at least partially, caused by a selection for translational efficiency [32].

Akashi (1994) examined the codon usage of 38 homologous genes from *Drosophila melanogaster*, *D. pseudoobscura,* and *D. virilis* and found that in genes with weak codon bias, the conserved amino acids had higher codon bias than the non-conserved residues [33]. In regions encoding important protein motifs (homeodomains and zincfinger domains), the frequency of preferred codons was higher than in the remaining section of the gene, and it was suggested that selection for translational accuracy caused this bias [34].

Here we analyzed the synonymous codon usage bias in two insect species belonging to the order Lepidoptera. In this study, we found that the most of the frequent codons end with C in both the species. This finding may be the result of compositional constraint that occurred in codon usage pattern in insect species. In *B. mandarina,* the ENC value ranged from 51 to 60 with a mean value of 57.75 and in *O. furnacalis,* from 52 to 60 with a mean value of 57.17 respectively. Relatively high ENC values showed that codon bias was not remarkable in these species. *B. mandarina* genes had greater gene expression than *O. furnacalis* genes. In both the species a highly significant positive correlation was observed between A% and A3% , C% and C3% , GC% and GC3%. In addition, a significant positive correlation between T% and T3%, positive correlation between G% and G3% and a negative correlation between GC% and T3% were observed. These results suggest that mutation pressure had been a major factor for codon usage bias in these insect species.

Conclusion

This is the first work on comparative analysis of the pattern of codon usage in two insect species of lepidoptera order. This work is useful for understanding the evolution of the pattern of codon usage in these species. Codon usage bias was not very remarkable in this species. Nucleotide constraint and compositional constraint are significant factors that affect codon usage pattern in these species. The most frequent codons end with C at the 3rd position, most probably suggesting the role of the compositional constraint under mutation pressure. We find that mutation pressure is the main factor for the pattern of codon usage bias. However further analysis is needed for understanding the role of any other factor that might be responsible for codon usage bias in insects. However further analysis is needed for elucidating the role of any other factor responsible for codon usage bias.

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Conflict of interest: This research work does not have any competing interest.

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Table. 9 Correlation coefficients between overall nucleotide composition (A %, T %, G %, C%, GC%) and nucleotide composition at 3rd position (A3 %, T3 %, G3% ,C3%, GC3%) in *Bombyx mandarina* genes

Nucleotide	A3 %	T3 %	G3 %	C3 %	GC3 %
A %	0.608**	0.444	-0.116	-0.648**	-0.641**
T %	0.808**	0.741**	-0.311	-0.871**	-0.859**
G %	-0.620**	-0.697**	0.575**	0.586**	0.763**
C %	-0.737**	-0.462*	0.009	0.844**	0.699**
GC %	-0.773**	-0.657**	0.227	0.845**	0.818**

Table. 10 Correlation efficient between overall nucleotide composition (A %, T %, G %, C%, GC%) and nucleotide composition at 3rd position (A3 %, T3 %, G3% ,C3%, GC3%) in *Ostrinia furnacalis*

Nucleotide	A3 %	T3 %	G3 %	C3 %	GC3 %
A %	0.892**	0.773**	-0.506*	-0.914**	-0.791**
T %	0.764**	0.874**	-0.486*	-0.900**	-0.890**
G %	-0.736**	-0.916**	0.505*	0.828**	0.875**
C %	-0.826**	-0.785**	0.381	0.974**	0.812**
GC %	-0.819**	-0.897**	0.469	0.951**	0.895**

***, **, *** Significant at p=0.05, 0.01, 0.001 respectively**

LISTS OF FIGURES

Fig.1 Nucleotide composition (overall and at 3rd codon position)

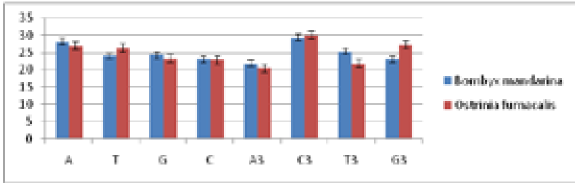


Fig. 2 Comparison of ENC in both species

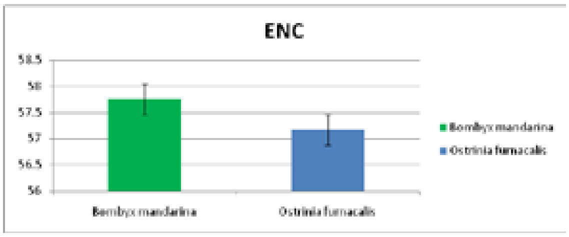


Fig. 3 Comparison of CAI in *B. mandarina* and *O. furnacalis*

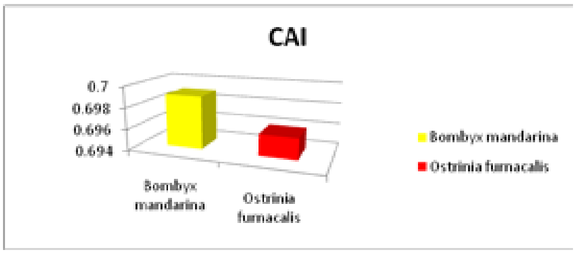


Fig. 4 Comparison of relative synonymous codon usage between *B. mandarina* and *O. furnacalis*

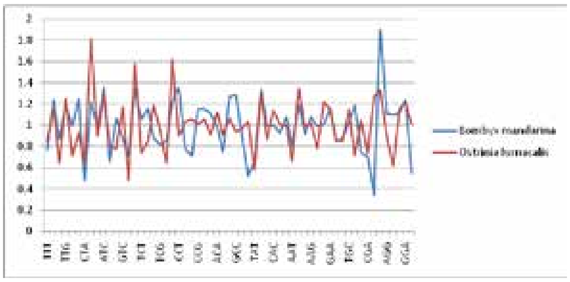


Fig. 5 Distribution of amino acids in different genes of *Bombyx mandarina (n= 20) and Ostrinia furnacalis (n=17)*

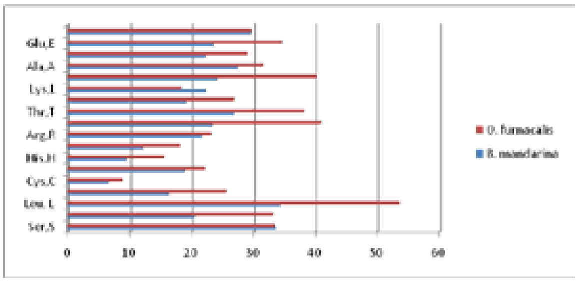


Fig. 6 Correlation between ENC and GC in *Bombyx mandarina*

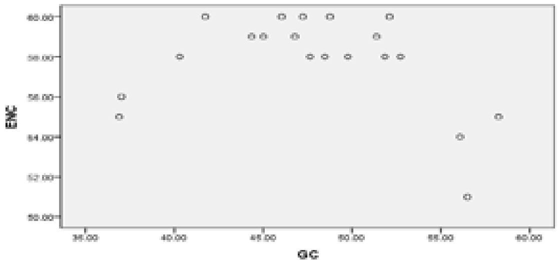
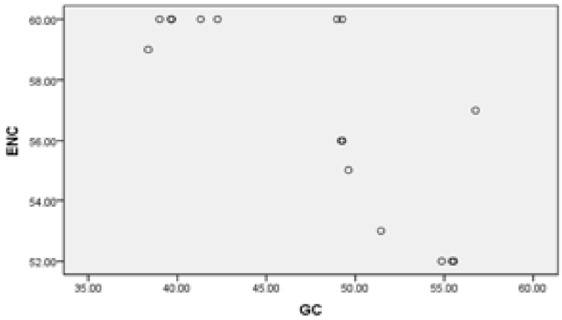


Fig. 7 Correlation between ENC and GC in *Ostrinia furnacalis*



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