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Natural Selection Shapes Codon Usage in the *Autographa californica* multiple nucleopolyhedrovirus Protein Kinase Gene

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INTRODUCTION & AIM

Baculoviruses are large double-stranded DNA viruses of the family *Baculoviridae* that cause lethal infections in insect populations, particularly in Lepidoptera, Diptera, and Hymenoptera. Due to their restriction to invertebrate hosts, they are widely used as biocontrol agents in integrated pest management [1]. Viral codon usage patterns provide valuable insights into evolutionary dynamics, gene expression regulation, and host adaptation.

Codon usage bias, defined as the preferential use of specific synonymous codons, plays a key role in gene evolution and protein expression efficiency [2]. Genome sequencing has identified open reading frames encoding serine/threonine protein kinases in fourteen baculovirus genomes. These viral protein kinases are involved in modulating host cell cycle processes, suppressing immune responses, and enhancing viral replication [3]. However, the codon usage bias of baculovirus protein kinase genes remains poorly characterized. Therefore, this study systematically analyzes codon usage patterns and the factors shaping them in protein kinase genes from fourteen baculoviruses, providing new insights into their functional and evolutionary properties.

METHOD

1. Sequence Data and Nucleotide Composition Analysis

Protein kinase gene sequences from fourteen baculoviruses were retrieved from the NCBI GenBank database. Nucleotide composition parameters were calculated using CodonW v1.4.2. These included overall nucleotide frequencies (A%, T%, C%, G%), GC content at different codon positions (GC1s, GC2s, GC12s, and GC3s), nucleotide usage at the third codon position (A3s%, T3s%, C3s%, G3s%), and total AT and GC contents. CAI values were calculated using the CAIcal server to estimate potential gene expression levels and viral adaptation to host codon usage preferences.

2. Relative Synonymous Codon Usage (RSCU)

RSCU values were calculated using CodonW v1.4.2 to assess synonymous codon usage patterns. Codons with RSCU >1.0 were considered preferentially used, whereas those with RSCU <1.0 were considered underused. Codons with RSCU ≥1.6 and ≤0.6 were classified as over- and under-represented, respectively [4].

3. Effective Number of Codons (ENC) and ENC-Plot

ENC values were computed using CodonW to quantify codon usage bias, with lower ENC values indicating stronger bias. ENC-plot analysis was performed by plotting ENC against GC3s to evaluate the relative contributions of mutation pressure and natural selection.

4. Neutrality Plot Analysis

Neutrality plots were generated by regressing GC3s against GC12s to assess the balance between mutation pressure and natural selection in shaping codon usage bias.

5. Parity Rule 2 (PR2) Plot Analysis

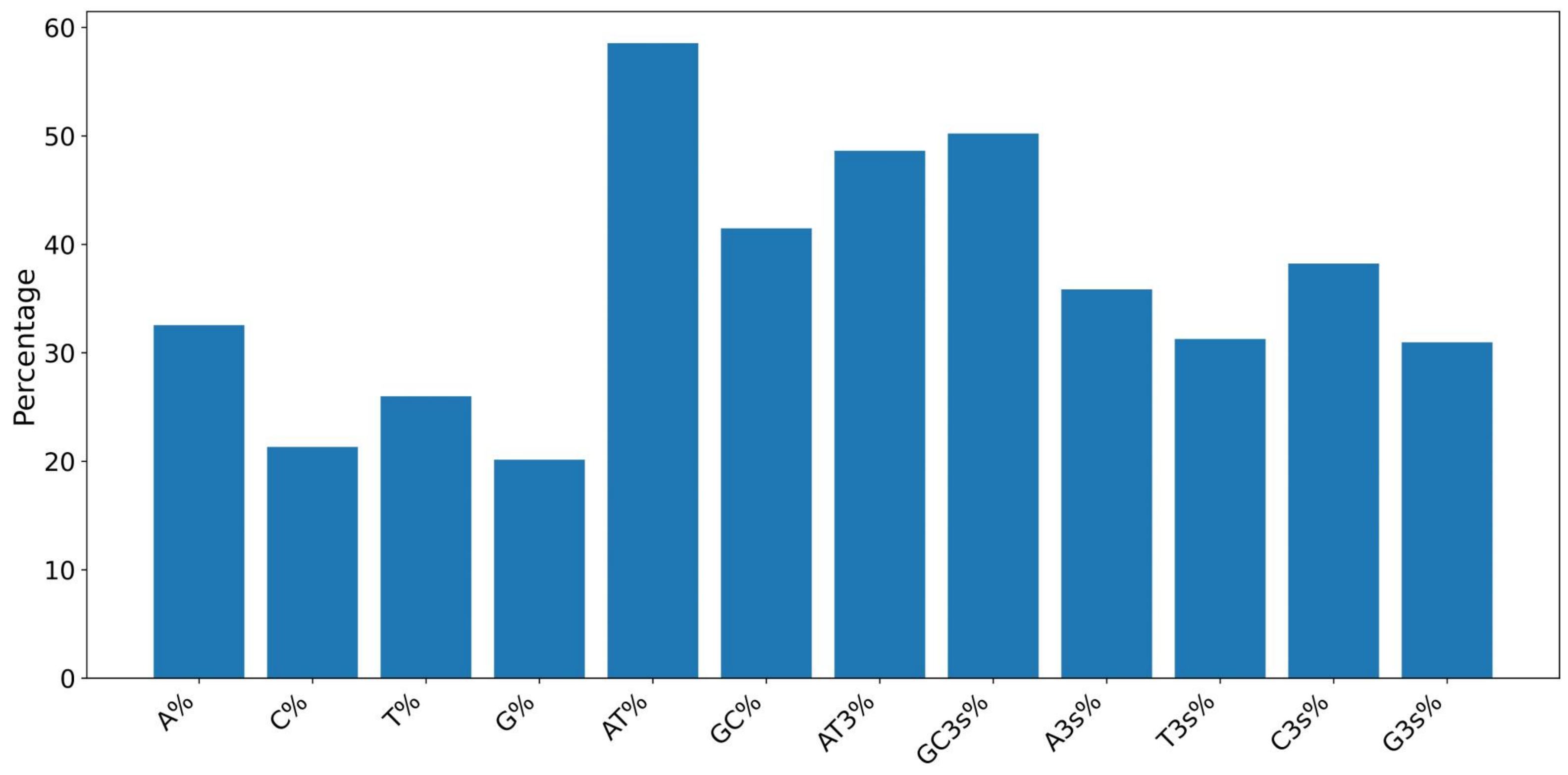
PR2 plots were used to examine nucleotide usage bias at the third codon position by plotting A3/(A3+T3) against G3/(G3+C3), allowing evaluation of the combined effects of mutation and selection.

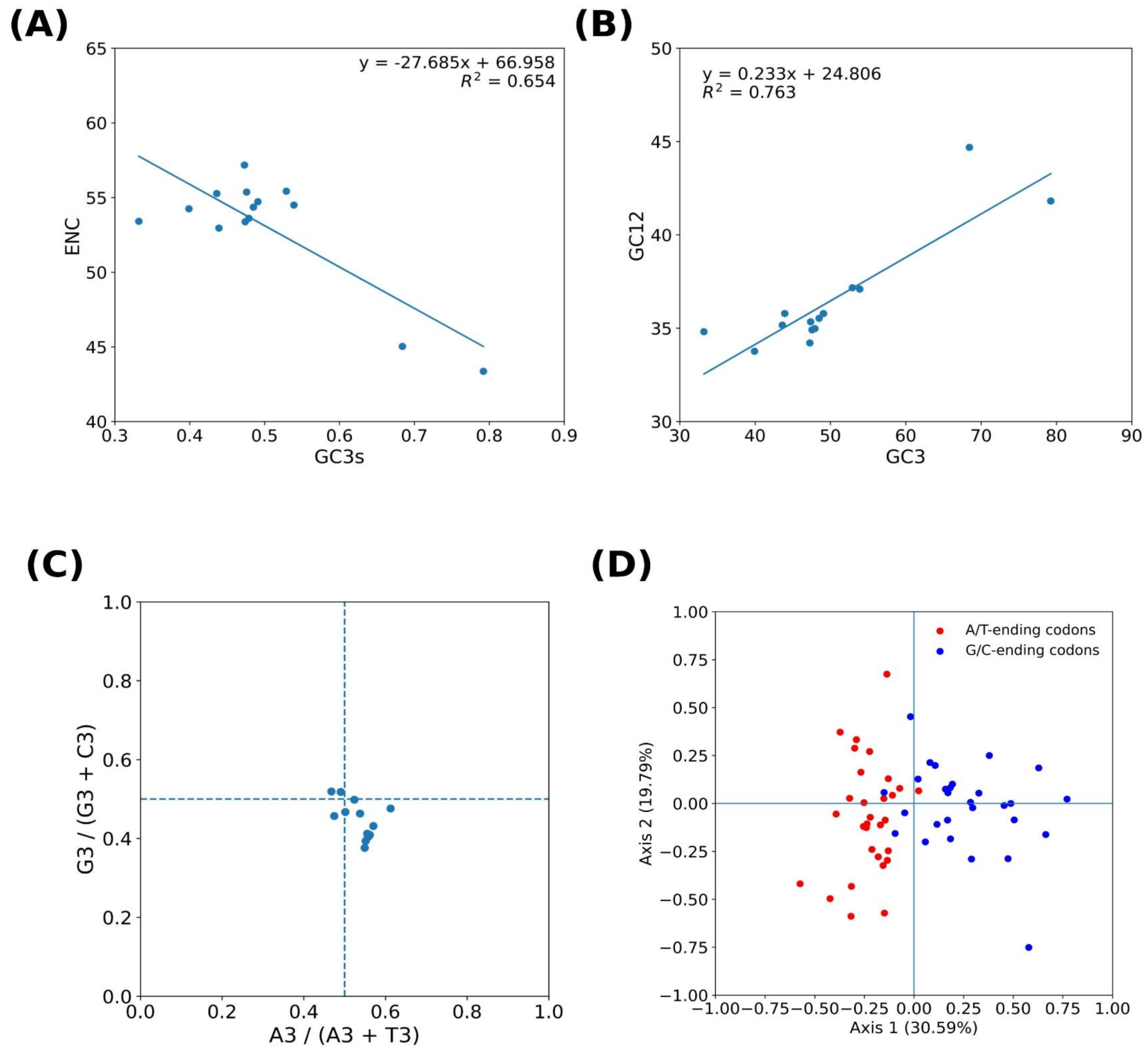
6. Correspondence Analysis (COA)

COA based on RSCU values was performed using CodonW to explore major trends in codon usage variation. Stop codons and Met and Trp codons were excluded, and the first two principal axes were used for visualization.

RESULTS & DISCUSSION

Results showed a strong preference for G- and C-ending codons. The AcMNPV PK gene exhibited low codon usage bias, while CAI analysis indicated high host adaptability.





ENC–GC3s plots (A), neutrality (B), and PR2 bias (C) analyses indicated that codon usage is mainly shaped by natural selection, with a minor contribution from mutation pressure. Correspondence analyses (D) further supported the combined effects of selection and mutation.

CONCLUSION

This study provides the first codon usage analysis of the *Autographa californica* multiple nucleopolyhedrovirus protein kinase gene. The PK gene shows a preference for G/C-ending codons and low codon usage bias. Natural selection is the primary force shaping codon usage, while mutation pressure plays a minor role. These findings offer new insights into the molecular evolution and regulation of baculovirus protein kinase genes.

FUTURE WORK / REFERENCES

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