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# Understanding Genetic Diversity of *Episyrphus balteatus* (De Geer, 1776) From Different Regions of Himachal Pradesh On the Basis of Designed COI Primer

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## Abstract

*During present investigation, a total nine localities i.e. Darlaghat (1563m), Jatoli (1464m), Kotla Panjola (1190m), Chandol (1418m), Dhar (1360 m), Alsindi (1132 m), Naldehra (2050 m), Ghanahatti (1668 metres) and Potters hill (1887 m) of Punica granatum were selected for the field surveys of insect pollinators from which Episyrphus balteatus was observed at four localities of Himachal pradesh i.e. Dhar (1360 m), Ghanahatti (1668 m), Kotla Panjola (1190m) and Naldehra (1887 m) and samples of species were collected. All the sampled species of Episyrphus balteatus from four localities were characterized genetically through mtCOI gene for the intraspecific diversity studies. Multiple sequence alignment between all the mtCOI sequences of Episyrphus balteatus revealed the similar pattern between the nucleotides of all the sequences. Nucleotide content analysis of all the species at first, second and third codon position revealed the average A+T percentage (69.96%) found to be higher than C+T (30.13%). The A+T bias was pronounced in general for this region for all codon positions. It was found that transition/transversion value of COI (R) is 0.00 which concluded that there is insignificant neutral selection in samples of Episyrphus balteatus of Himachal Pradesh. Phylogenetic analysis studies showed the sample of Naldehra and Ghanahatti were found to be closely related to each other and of Dhar and Kotla Panjola samples were also phylogenetically close to each other. Distance matrix also clearly indicates the very less difference among the sampled species proves that there is no genetic diversity between them.*

## 1. Introduction

Pollination is a very important ecosystem service for reproduction of most flowering plants and pollinating animals are necessary for transmitting genes within and among populations of wild plant species [1]. Although most scientific research has concentrated on pollination problems in wild plants but animal pollination has become increasingly important in food production in recent years. According to [2], animal pollination is required for the production of fruit, vegetables, and seeds from 87 of the world's most important food crops, accounting for 35 % of worldwide food output. gave a complete list for 1 330 tropical plant species, demonstrating that animal pollination improves at least one variety of tropical crops in almost 70% of cases [3]. Also highlighted that flower visiting insects provide an important ecosystem function to worldwide agricultural production through pollination services [4].

Although insect pollinators are very crucial for the ecosystem maintenance, but they are negatively threatened as a result of multiple environmental pressures [5]. Plant-pollinator interactions have been shown to be negatively affected by invasive species [6, 7] pesticide use [1,8] climate change, land-use changes such as habitat fragmentation [9,10,11] and agricultural intensification [12, 13].

Now a days, Global climate change also a serious threat to insect pollinators and pollination services [6,5,14, 15] proposes three scenarios for species reactions to large-scale climate change i.e. adaptation to new environment, Emigration to another suitable area and Extinction. Climatic change will occur too quickly for populations to adjust through genetic changes. The distributions of species are projected to shift towards the poles and higher altitudes when temperatures rise and exceed their thermal tolerance thresholds [16,14]. As a result of climate change, many studies have discovered poleward expansions of plants

[17,18,19,20] and butterflies [21,22]. Crop species and pollinators that have been managed can be easily transported and grown in more favorable environments. On the other hand, moving food production to other places could have major socio-economic implications. Furthermore, wild pollinators may be unable to track the movement of crops. Due to climate change, if insect pollinators get affected and decline, definitely species which are dependent on the pollinators will also show decrease in their diversity as well as in crop pollination because these species have strong biotic interaction between them.

Wild pomegranate (*Punica granatum L.*) is medicinally very important wild fruit crop of Himachal Pradesh [23] and it is well known for its medicinal properties. Various types of polyphenols primarily ellagic and punicalagin has been found in the pomegranate juice that may reduce the risk of heart diseases [24] and also slow down the cancer progress (Adams *et al*., 2006). Its fruits helpful in the treatment of vomiting, sore throat, brain diseases, spleen complains, bronchitis, liver and kidney disorders [25]. Due to its medicinal properties, the wild pomegranate germplasm in Himalayan region is eroding fast due to human incursion [26,27] and pollinators diversity were also declining day by day due to such human activities.

So, keeping in view the role of wild pollinators to increase production of wild crops including fruits, vegetables etc., the present investigations were conducted on genetic diversity of *Episyrphus balteatus.* This study will be preliminary or base model for further investigation, so the researches can carry study regarding their role of this species to increase the production and diversity of *Punica granatum* in different areas of Himachal Pradesh and further investigation can be carried out on conservation, monitoring and management of *Punica granatum* as well as *Episyrphus balteatus* in the Himachal Pradesh.

## 2. Material and Methods

During a study period, total 9 localities i.e. Darlaghat (1563m), Jatoli (1464m), Kotla Panjola (1190m), Chandol (1418m), Dhar (1360 m), Alsindi (1132 m), Naldehra (2050 m), Ghanahatti (1668 metres) and Potters hill (1887 m) of *Punica granatum* were selected for the field surveys of insect pollinators from which *Episyrphus balteatus* was observed at four localities i.e. Dhar (1360 m), Ghanahatti (1668 metres), Kotla Panjola (1190m) and Naldehra (1887 m) and samples of species were collected. Collected specimens were preserved immediately in refrigerator at -800C until DNA extraction. DNA was extracted from the thorax or upper abdominal region of the insect specimen by using DNeasy blood and tissue Qiagen Kit method by following standardized protocol of the manufacturers. Extracted DNA was preserved in the -200C for further use. Target DNA from mitochondrial gene, i.e. Cytochrome Oxidase subunit I was amplified using a pair of forward primers LCO1490 5'- GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3' and reverse primer HCO2198 5'- TAA-ACT-TCA-GGG-TGACCA-AAA-AAT-CA-3' [28]. PCR reaction was performed in 96-well plates with 20 µl reaction volume containing 1µL DNA template; 1 µL primer forward; 1 µL primer reverse; 5 µL distilled water; 12 µL Emerald PCR master mix in a C1000™ Thermal Cycler. Thermocycling consisted of a pre-denaturation at temperature of 940C for 4 minutes followed by 30 cycles with denaturation reaction conditions at temperature of $94^0$C for 40 seconds, annealing at temperature of $50^0$C for 30 seconds, and extension at temperature of $72^0$C for 50 seconds. Then process of PCR ended with final extension at 720C temperature for 6 minutes.

The amplified product was analysed on a 1.2% agarose gel electrophoresis and checked under UV light and documented. The amplified DNA fragments were extracted from agarose gels and purified using DNA/RNA purification Qiagen Kit method standardized by manufacturers. The primers used were the same primers used in PCR amplification and sequencing was done in "Big dye terminator version 3.1" cycle sequencing kit with sequencing machine–ABI 3500xL Genetic analyser. After completion of sequencing, all fasta format sequences obtained by Sanger sequencing was used for BLAST search to check the sequence homology at NCBI. All the sequences were edited and aligned using bioedit sequence alignment editor software. All the gaps and mismatched were removed and sequences were submitted in the gene bank for accession Number (Table 1).

| S. No. | Species name | Sample Location | Geographical Location | | | Genebank Accession Number |
|---|---|---|---|---|---|---|
| | | Locality | Longitude | Latitude | Altitude | COI |
| 1 | *Episyrphus balteatus* | Dhar | 76°-82′85 | 31°-62′42 | 1360 m | OL305829 |
| 2 | *Episyrphus balteatus* | Ghanahatti | 77°-05′04 | 31°-08′18 | 1668 m | OL405702 |
| 3 | *Episyrphus balteatus* | Kotla Panjola | 77°-08′51 | 30°-51′09 | 1190 m | OK655768 |
| 4 | *Episyrphus balteatus* | Naldehra | 77°-18′69 | 31°-18′39 | 1887 m | OL765264 |

**Table 1: Places of sample collection of *Episyrphus balteatus* with geographical location and Genbank accession numbers of COI gene.**

The nucleotide content (A, T, G, C) of all the samples and the total C+G and A+T at first, second and third codon position were calculated using MEGA X software (Table 3). DNADIST with the Kimura two parameter distance option was used to estimate divergence between sequences with a transition/transversion ratio in the MEGA X software. In this study, phylogenetic analysis of obtained 4 sequences of sampled *Episyrphus balteatus* were conducted using Neighbor-Joining method and Kimura-2 parameter in MEGA X. Sequences were aligned using the MEGA X software [29]. Analyses were performed on 1000 bootstrapped data sets generated by the program [30].

## 3. Results and Discussion

DNA was extracted from the collected samples of *Episyrphus balteatus*. MtDNA COI gene was effectively amplified for 710 bases (Fig.1) and sequenced by Sanger sequencing method. Sequences were checked in NCBI-BLAST to confirm the species similarity, which matched with previous submitted mtCOI sequences of *Episyrphus balteatus* in NCBI with 98 to 100%. Sequences were submitted to genebank and accessed with accession numbers from genebank (Table 1). All the DNA sequences were aligned using multiple sequence alignment program CLUSTAL Omega in which alignment between all the mtCOI sequences of *Episyrphus balteatus* revealed the similar pattern between the nucleotides of all the sequences (Fig. 2).

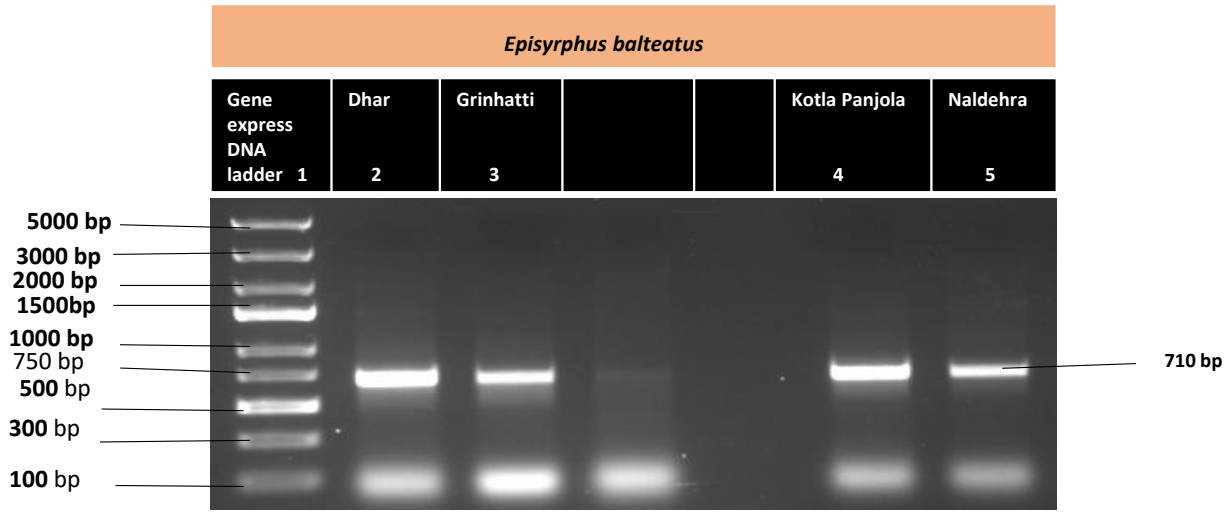


**Figure 1:** Analysis of Amplified PCR Product in 1.2% Agarose, Lane 1: Gene ruler express DNA ladder, Lane 2,3,4,5,6: 710 bp size mtCO1 gene

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CLUSTAL O(1.2.4) multiple sequence alignment


OK655768      TTCGGAGCTTGAGCTGGAATAGTAGGTACATCATTAAGTGTATTAATTCGTGCAGAACTT   60
OL305829      TTCGGAGCTTGAGCTGGAATAGTAGGTACATCATTAAGTGTATTAATTCGTGCAGAACTT   60
OL405702      ---------TGAGCTGGAATAGTAGGTACATCATTAAGTGTATTAATTCGTGCAGAACTT   51
OL765264      ----------------------AGAAGGTACATCATTAAGTGTATTAATTCGTGCAGAACTT   40
                                    ** ************************************

OK655768      GGTCATCCTGGTGCTTTAATTGGAGATGATCAAATTTATAATGTAATTGTTACAGCCCAT   120
OL305829      GGTCATCCTGGTGCTTTAATTGGAGATGATCAAATTTATAATGTAATTGTTACAGCCCAT   120
OL405702      GGTCATCCTGGTGCTTTAATTGGAGATGATCAAATTTATAATGTAATTGTTACAGCCCAT   111
OL765264      GGTCATCCTGGTGCTTTAATTGGAGATGATCAAATTTATAATGTAATTGTTACAGCCCAT   100
              ************************************************************

OK655768      GCTTTTGTAATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGA   180
OL305829      GCTTTTGTAATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGA   180
OL405702      GCTTTTGTAATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGA   171
OL765264      GCTTTTGTAATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGA   160
              ************************************************************

OK655768      TTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGTTTAAATAATATAAGT   240
OL305829      TTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGTTTAAATAATATAAGT   240
OL405702      TTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGTTTAAATAATATAAGT   231
OL765264      TTAGTTCCATTAATATTAGGAGCTCCTGATATAGCATTTCCTCGTTTAAATAATATAAGT   220
              ************************************************************
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**Figure 2:** CLUSTAL O (1.2.4) Multiple sequence alignment of COI Sequences of *Episyrphus balteatus* from different areas of Himachal Pradesh

**Nucleotide content analysis of COI gene:** According to the neutral theory, nucleotide polymorphism levels correlate to evolutionary rate, and the transition and transversion ratio within populations should be related to long-term evolutionary rate.

**Comparative significance of Transitions and Transversions:** The frequencies of transitions and transversions is mentioned in Table 2. The estimated transition/transversion (Ts/Tv) bias of COI (R) is 0.00. The percentage of sites showing transitions (0%) is less than the number of sites showing transversions. The nucleotide frequencies are 29.63% (A), 40.30% (T/U), 13.87% (C), and 16.20% (G).

| **Transitions (%)** | | | | | **Transversions (%)** | | | | | | | | **Ts/Tv ratio** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **COI Gene** | G/A | C/T | T/C | A/G | A/T | A/C | T/A | T/G | C/A | C/G | G/T | G/C | 0.00 |
| | 0.00 | 0.00 | 0.00 | 0.00 | 20.15 | 6.93 | 14.82 | 8.1 | 14.82 | 8.1 | 20.15 | 6.93 | |

**Table 2: Frequency percentage (%) of transitions and transversions and transition/transversion ratio (Ts/Tv) of COI gene.**

### 4. Base composition at each Codon positions

The nucleotide content (A,T,G,C) and the total C+G and A+T at first, second and third codon position of all the samples revealed the high numbers of polymorphic sites verified in the COI gene were evenly distributed among the 3 codon positions. Average A+T percentage (69.96%) found to be higher than C+T (30.13%) (Table 3). The A+T bias was pronounced in general for this region for all codon positions

| Samples | First codon | | | | Second codon | | | | Third codon | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Episyrphus balteatus*** | **A** | **C** | **G** | **T** | **A** | **C** | **G** | **T** | **A** | **C** | **G** | **T** | **C+G** | **A+T** |
| OL305829, Dhar | 25.4 | 14.6 | 32.4 | 27.7 | 14.6 | 25.8 | 17.4 | 42.3 | 48.8 | 1.4 | 0.0 | 49.8 | 30.53 | 69.53 |
| OK655768, Kotla Panjola | 25.2 | 14.5 | 32.2 | 28.0 | 14.5 | 25.7 | 17.3 | 42.5 | 48.6 | 1.4 | 0.0 | 50.0 | 30.36 | 69.60 |
| OL765264, Naldehra | 25.2 | 14.8 | 31.0 | 29.0 | 15.2 | 25.2 | 16.7 | 42.9 | 48.3 | 0.9 | 0.0 | 50.7 | 29.53 | 70.43 |
| OL405702, Grinhatti | 25.0 | 14.8 | 31.5 | 28.7 | 14.4 | 25.6 | 17.2 | 42.8 | 48.4 | 0.9 | 0.0 | 50.7 | 30.1 | 70.00 |
| Average | 25.2 | 14.7 | 31.8 | 28.4 | 14.7 | 25.6 | 17.1 | 42.8 | 48.5 | 1.2 | 0.0 | 50.3 | 30.13 | 69.96 |

**Table 3: Mean frequencies (%) for base compositions at different codon positions for COI region.**

In determining the degree and direction of natural selection, the transition/transversion (Ts/Tv) ratio is important. Transitions do not contribute significantly to genetic divergence, whereas transversions have a considerable impact on species evolution. Present study revealed that the transition/transversion value of COI (R) is 0.00. The values of transition/transversion ratio in the present study does not indicates any genetic diversity between species but there is possibility of genetic divergence in *Episyrphus balteatus* of Himachal Pradesh over evolutionary time scale.

### 5. Phylogenetic analysis of mitochondrial COI of *Episyrphus balteatus* samples

Phylogenetic relationship among *Episyrphus balteatus* of the present study obtained through Neighbor- Joining method which showed that among the four samples sequences, the sample of Naldehra and Ghanahatti were found to be closely related to each other and Dhar and Kotla panjola samples were also phylogenetically very close to each other. Distance matrix also clearly indicates the very less difference among the sampled species proves that there is no genetic diversity between them (Fig. 3).

Results are very much similar with the findings of Willis *et al.* (1992), who also found that honeybees are AT-biased and average A+T and C+G content of the findings in the ratio of 3:1 respectively. Similarly, Chalapathy et al. (2014) also studied the mitochondrial DNA diversity of *Apis cerana* populations of Nilgiri Biosphere Reserve. They characterized bee colonies from 10 localities of Nilgiri Biosphere genetically through COI gene of mitochondrial genome. They also observed that nucleotide composition of the mtCOI of Apis cerana were also strongly biased toward A and T with an average AT content of 75.6%. In 2019, Gaikwad also investigated phylogenetic variations in Apis cerana from Maharashtra's North Western Ghats.

Due to great variations in elevation and climatic conditions, Himachal Pradesh is one of the world's richest reservoirs of biological diversity. The world's biodiversity is under constant threat from environmental change. Species must adapt to a continually changing environment in order to live. As a result, adaptation to environmental changes becomes vital in order to avoid extinction [31]. Recent research suggests that global climate change may have an impact on the genetic diversity of stationary populations. The evolutionary adaptive capacity of a species is determined by micro-evolution, which includes selection for local genotypes better adapted to changing environmental conditions [32] as well as the evolution of phenotypic plasticity [33, 34]. The current study, which is limited to a few Western Himalayan locations, indicates less genetic difference. However, a comprehensive survey of Episyrphus balteatus across Himachal Pradesh would yield fascinating insights on the species genetic diversity.

### 6. Conclusion

Evolutionary impacts of global climate change altered intraspecific genetic diversity. It causes variations in phenotypic plasticity levels in individuals and communities as they respond to changing environmental conditions. These changes also reduce genetic diversity in populations and species, perhaps resulting in population viability and extinction. To reduce the risk of extinction of species and ecosystems, widespread sampling and deeper characterization of genetic diversity with diverse mitochondrial genes is essential.

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