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Research Article

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A Study on Genetic Diversity of Eristalis Tenax(Linnaeus, 1758) From Different Areas of Western Himalaya

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Abstract

During a study period, total 9 localities i.e.Darlaghat (1563m), Jatoli (1464m), KotlaPanjola (1190m), Chandol (1418m), Dhar (1360 m), Alsindi (1132 m), Naldehra (2050 m), Ghanahatti (1668 metres) and Potters hill (1887 m)of Punicagranatum were selected for the field surveysfrom which Eristalis tenax was observed on the Punicagranatumatfour localitiesi.e. Darlaghat (1563 m), Naldehra (1887 m), KotlaPanjola (1190 m) and Pottershill (1418 m) and samples of species were collected.All the sampled species of Eristalis tenax four localities were characterized genetically through mtCOI gene for the intraspecific diversity studies. Multiplesequence alignment between all the mtCOI sequences of Eristalis tenaxrevealed 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 theaverage A+T percentage (69.14%) was higher than C+T (30.86%). The A+T bias was prominent for all codon sites. Itwas found that transition/transversion value of COI (R) is 0.50 which concluded that there is insignificant neutral selection in samples of Eristalis tenax of Himachal Pradesh. Phylogenetic analysis studies showed that the sample of Naldehra and Darlaghat were phylogenetically similar to each other and the sample of Pottershilland Darlaghat were found to be quite dissimilar from each other.

Keywords: Punicagranatum; Eristalis tenax;mtCOI; Genetic Diversity.

Introduction

Wild pollinating species play very important and valuable role in crop pollination services [1]. Around 80% of food plants worldwide rely on insect pollinators for pollination or their yield increased by them [2]. Among insect species, bees pollinate 92% of all crops worldwide, whereas flies are the second most significant flower visitors [3, 4, 5, 6].Flies can pollinate some crops more efficiently or better than bees [7, 8,9,10]and are frequently responsible for carryinglargepollen loads in both natural and modified systems [11, 10]. Many of these "unmanaged pollinators" and "exotic flies" can thus reliably pollinate specific crops [8, 12, 13].Flies belong to the order Dipterawhich comprises about 150 000 species, including over 6000 hoverflies (Syrphidae), which are divided into 209 genera [14]. Among syrphidae, genus *Eristalis* can be found all over the world [15, 16, 17, 18, 19, 20, 21, 22].

Hoverflies, like bees and bumblebees, are equally important pollinators [8, 23, 24, 25]. But unfortunately, pollinator populations have been declining at an alarming rate (Hallmannet al. 2017). In 2018, the European Union Pollinators Initiative reported that all types of European pollinators including hoverflies are in decline [26]. Habitat degradation, pollution, climate change, over-exploitation and environmental variation threatened the extinction of many of these pollinators. The majority of these are caused by human activity.

Climate change is a major issue now a days which severely affecting insect pollinators. Pollination services could be threatened even more by climate change [27, 28, 29]. Climate change poses a threat not only to individual species, but also to the genetic diversity that exists within them. Due to this reason, many wildlife populations have been reduced to small isolated fragmented groups with lower survival capacity. Small populations are potentially at risk and the main goal to study genetic diversity is to apply the knowledge of genetics to reduce the risk of extinction [30]. Genetic diversity help to maintain the higher levels of biodiversity (populations, species) and it is the foundation of a species evolutionary potential to adjust to environmental changes, making it an important pillar in conservation genetics [31]. The impact of climate change on pollinators is determined by their thermal tolerance and temperature plasticity. Some of species are better adapted to warmer climates and may thus expand into new places where they can serve

as pollinators in the future climatic conditions [32, 29].

Mitochondrial gene, Cytochrome oxidase I is one of the molecular marker used to resolve the phylogeny in several taxa [33, 34]. becausemtDNAis transmitted maternally without recombination and has a high rate of intraspecific variation [35]. Mitochondrial DNA (mtDNA) is most important and frequently used molecule in systems biology, species characterization, population structure and phylogenetic research. Mitochondrial DNA has been widely used in phylogenetic investigations of *Apismellifera*[36, 37,38,39, 40, 41, 42] but studied more rarely for *Eristalis tenax*.

Keeping the above in view, an attempt has been made to study the genetic diversity of of *Eristalis tenax*, most important pollinator of wild pomegranate in Western Himalaya, through molecular tools and characterize the relationships Himalayas*alistenax* from different areas of Western Himalaya.

Material and Methods

During a study period, total 9 localities i.e.Darlaghat (1563m), Jatoli (1464m), KotlaPanjola (1190m), Chandol (1418m), Dhar (1360 m), Alsindi (1132 m), Naldehra (2050 m), Ghanahatti (1668 metres) and Potters hill (1887 m) of *Punicagranatum* were selected for the field surveys from which *Eristalis tenax* was observed on the *Punicagranatum* at four localitiesi.e. Darlaghat (1563 m), Naldehra (1887 m), KotlaPanjola (1190 m) and Pottershill (1418 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-TGAC-CA-AAA-AAT-CA-3' (Folmeret al. 1994). 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 an pre-denaturation at temperature of 940C for 4 minutes followed by 30 cycles with denaturation reaction conditions at temperature of 940C for 40 seconds, annealing at temperature of 500C for 30 seconds, and extension at temperature of 720C 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 a sequencing machine–ABI 3500xL Genetic analyser. After completion of sequencing, all fasta format sequences obtained by Sanger sequencingwas 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).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 Eristalis tenax were conducted using Neighbor-Joining method and Kimura-2 parameter in MEGA X.Sequences were aligned using the MEGA X software [43].Analyses were performed on 1000 bootstrapped data sets generated by the program [44].

Table 1: Localities of sample collection of *Eristalis tenax* with geographical location and the Genbank accession numbers of COI gene.

S. No.	Species name	Sample Location	Geographical Location			Genebank Accession Number
		Locality	Longitude	Latitude	Altitude	COI
1	Eristalis tenax	Darlaghat	76°-56′50	31°-13′14	1563 m	OK465131
2	Eristalis tenax	Naldehra	77°-18′69	31°-18′39	1887m	OL441830
3	Eristalis tenax	KotlaPanjola	77°-08′51	30°-51′09	1190 m	OM618002
4	Eristalis tenax	Pottershill	77°-34′37	30°-93′60	1418 m	OM570334

Results and Discussion

Samples of *Eristalis tenax* were collected from four different areas of Western Himalayas and DNA extraction was performed. The mtDNA COI gene was effectively amplified for 710 bases in *Eristalis tenax* samples(Fig. 1) and sequenced by Sanger sequencing method. Sequences were checked in NCBI-BLAST to confirm the species similarity, which matched with previous submitted sequences of *Eristalis tenax* 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 Omegain which alignment between all the mtCOI sequences of *Eristalis tenax* revealed the similar pattern between the nucleotides of all the sequences(Fig. 2).

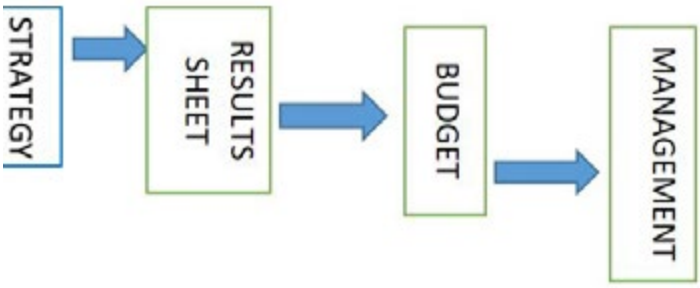

Figure 1: Analysis of amplified PCR product in 1.2% agarose: Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5: 710 bp size mtCOI gene

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OK465131  ------------------------------------------------------------0
OL441830  ACATGAGCAGGTATAGTAGGAACTTCCTTA-
AGAATTTTAATTCGAGCTGAATTAGGCCAT  60
OM618002  -------------------GAACTTCCTTAAGAATTTTA-
ATTCGAGCTGAATTAGGCCAT  41
OM570334  --------AGGTATAGTAGGAACTTCCTTA-
AGAATTTTAATTCGAGCTGAATTAGGCCAT  52

OK465131  ------GCATTAATTGGAGATGATCAAATTTATA-
ATGTTATTGTAACAGCTCATGCCTTT  54
OL441830  CCTGGAGCATTAATTGGAGATGAT-
CAAATTTATAATGTTATTGTAACAGCTCATGCCTTT
120
OM618002  CCTGGAGCATTAATTGGAGATGAT-
CAAATTTATAATGTTATTGTAACAGCTCATGCCTTT
101
OM570334  CCTGGAGCATTAATTGGAGATGAT-
CAAATTTATAATGTTATTGTAACAGCTCATGCCTTT
112
          ****************************************
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OK465131  GTAATAATTTTCTTTATAGTAATACCTATTATA-
ATTGGAGGATTTGGAAATTGATTAGTT 114
OL441830  GTAATAATTTTCTTTATAGTAATACCTATTATA-
ATTGGAGGATTTGGAAATTGATTAGTT 180
OM618002  GTAATAATTTTCTTTATAGTAATACCTATTATA-
ATTGGAGGATTTGGAAATTGATTAGTT 161
OM570334  GTAATAATTTTCTTTATAGTAATACCTATTATA-
ATTGGAGGATTTGGAAATTGATTAGTT 172
          ****************************************
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OK465131  CCTCTTATATTAGGAGCCCCTGA-
TATAGCATTTCCTCGAATAAATAATATAAGTTTCTGA
174
OL441830  CCTCTTATATTAGGAGCCCCTGA-
TATAGCATTTCCTCGAATAAATAATATAAGTTTCTGA
240
OM618002  CCTCTTATATTAGGAGCCCCTGA-
TATAGCATTTCCTCGAATAAATAATATAAGTTTCTGA
221
OM570334  CCTCTTATATTAGGAGCCCCTGA-
TATAGCATTTCCTCGAATAAATAATATAAGTTTCTGA
232
          ****************************************
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OK465131  TTATTACCTCCTTCTTTAACATTATTATTAGTA-
AGAAGTATAGTAGAAAATGGAGCTGGA  234
OL441830  TTATTACCTCCTTCTTTAACATTATTATTAG-
TAAGAAGTATAGTAGAAAATGGAGCTGGA  300
OM618002  TTATTACCTCCTTCTTTAACATTATTATTAG-
TAAGAAGTATAGTAGAAAATGGAGCTGGA  281
OM570334  TTATTACCTCCTTCTTTAACATTATTATTAG-
TAAGAAGTATAGTAGAAAATGGAGCTGGA  292
          ****************************************
**************

OK465131  ACAGGATGAACTGTATACCCACCTTTAT-
CAAGTAACATTGCACACGGAGGAGCCTCAGTT
294
OL441830  ACAGGATGAACTGTATACCCACCTTTAT-
CAAGTAACATTGCACACGGAGGAGCCTCAGTT
360
OM618002  ACAGGATGAACTGTATACCCACCTTTAT-
CAAGTAACATTGCACACGGAGGAGCCTCAGTT
341
OM570334  ACAGGATGAACTGTATACCCACCTTTAT-
CAAGTAACATTGCACACGGAGGAGCCTCAGTT
352
          ****************************************
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OK465131  GATTTAGCAATTTTTTCACTTCATTTATCTG-
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GTATATCATCTATTTTAGGTGCAGTAAAT 354
OL441830 GATTTAGCAATTTTTTCACTTCATTTATCTGG-TATATCATCTATTTTAGGTGCAGTAAAT 420
OM618002 GATTTAGCAATTTTTTCACTTCATTTATCTG-GTATATCATCTATTTTAGGTGCAGTAAAT 401
OM570334 GATTTAGCAATTTTTTCACTTCATTTATCTG-GTATATCATCTATTTTAGGTGCAGTAAAT 412
********** **********

OK465131 TTCATTACAACTATTATTAATATACGATCAA-CAGGAATTACATATGATCGAATACCTTTA 414
OL441830 TTCATTACAACTATTATTAATATACGATCAA-CAGGAATTACATATGATCGAATACCTTTA 480
OM618002 TTCATTACAACTATTATTAATATACGATCAA-CAGGAATTACATATGATCGAATACCTTTA 461
OM570334 TTCATTACAACTATTATTAATATACGATCAA-CAGGAATTACATATGATCGAATACCTTTA 472
********** **********

OK465131 TTTGTATGATCTGTAGGTATTACAGCTTTAC-TACTACTTCTTTCCCTACCAGTTTTAGCA 474
OL441830 TTTGTATGATCTGTAGGTATTACAGCTTTAC-TACTACTTCTTTCCCTACCAGTTTTAGCA 540
OM618002 TTTGTATGATCTGTAGGTATTACAGCTTTAC-TACTACTTCTTTCCCTACCAGTTTTAGCA 521
OM570334 TTTGTATGATCTGTAGGTATTACAGCTTTAC-TACTACTTCTTTCCCTACCAGTTTTAGCA 532
********** **********

OK465131 GGAGCAATTACTATATTATTAACTGATC-GAAATTTAAATACATCATTTTTTGATCCAGCA 534
OL441830 GGAGCAATTACTATATTATTAACTGATC-GAAATTTAAATACATCATTTTTTGATCCAGCA 600
OM618002 GGAGCAATTACTATATTATTAACTGATC-GAAATTTAAATACATCATTTTTTGATCCAGCA 581
OM570334 GGAGCAATTACTATATTATTAACTGATC-GAAATTTAAATACATCATTTTTTGATCCAGCA 592
********** **********

OK465131 GGAGG---------- 539
OL441830 GGAGGAGGTGATCCAATTTTATACCAA-CATTTATTTTGATTTTTTGG 647
OM618002 GGAGGAGGTGATCCAATTTTATACCAA-CATTTATTTTGATTTTTT-- 626
OM570334 GGAGGAGGTGATCCAATTTTATACCAA-CATTTATTTTGATTTTTTG- 638

Figure 2: CLUSTAL O (1.2.4) multiple sequence alignment of COI sequences of Eristalis tenaxfrom different areas of Western Himalaya.

Nucleotide content analysis of COI gene: Under the neutral theory, nucleotide polymorphism levels should be associated with evolutionary rate and there should be a relationship between the transition and transversion ratio within populations and long-term evolutionary rate.

Comparative significance of Transitions and Transversions

The estimated transition/transversion (Ts/Tv) bias of COI (R) is 0.50 (Table 2). Present study showed that the percentage of sites showing transversions (66.67%)is higher than the number of sites showing transitions (33.34%)(Table 2)and nucleotide frequencies are 31.58% (A), 37.76% (T/U), 15.64% (C) and 15.02% (G). 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.

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

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	0.50
	10.53	12.59	5.21	5.01	12.59	5.21	10.53	5.01	10.53	5.01	12.59	5.21	

Base composition at each Codon positions

The nucleotide content (A,T,G,C) and total C+G and A+T at first, second and third codon position in the study also showed the high numbers of polymorphic sites in the COI gene which were evenly distributed among the 3 codon positions (Table 3). Average A+T percentage (69.14%) found to be higher than C+T (30.86%) (Table 3). For all codon sites, the A+T bias was prominent in this area. Nucleotide content of all the samples and total A+T and C+G were calculated by MEGA X software.

Transition/transversion (Ts/Tv) ratio helpful in assuming the degree and direction of natural selection. Positive or Darwinian se-

lection is indicated by a transition/transversion ratio greater than 1, purifying selection is shown by a ratio less than 1 and neutral selection is indicated by a ratio equal to one. Current study showed that transition/transversion value of COI (R) is 0.50. In this study, the estimated value of transition and transversion revealed that there is insignificant neutral selection in samples of *Eristalis tenax* ofWestern Himalaya. But there is possibility of genetic divergence in the *Eristalis tenax* of Western Himalayaover evolutionary time scale.

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

Samples	First codon				Second codon				Third codon				Total (%)	
Eristalis tenaxCOI	A	C	G	T	A	C	G	T	A	C	G	T	C+G	A+T
OM570334, Potterhill	28.2	15.0	29.1	27.7	14.6	25.0	17.5	42.9	51.6	6.1	0.0	42.3	30.9	69.1
OM618002, KotlaPanjola	28.4	15.4	27.9	28.8	14.8	25.4	17.2	42.6	51.2	6.2	0.0	42.6	30.7	69.3
OL441830, Naldehra	28.2	14.8	29.2	27.8	14.4	25.5	18.1	42.1	52.1	6.0	0.0	41.9	31.2	68.8
OK465131, Darlaghat	30.0	14.4	28.9	26.7	13.9	26.7	16.1	43.3	52.0	5.6	0.0	42.5	30.56	69.44
Average	**28.6**	**14.9**	**28.8**	**27.7**	**14.4**	**25.6**	**17.3**	**42.7**	**51.7**	**6.0**	**0.0**	**42.3**	**30.86**	**69.14**

Phylogenetic Analysis of Mitochondrial Coi of Eristalis Tenax Samples

Based on mitochondrial gene COI regions of four samples, the phylogenetic relationship was obtained through Neighbor-Joining method (Fig. 3). The high boostrap scores give confidence to support the separation of populations in well separated branch. Phylogenetic relationship showed that among the four sampled sequences, the sample of Naldehra and Darlaghat were phylogenetically similar to each other and the sample of Pottershill and Darlaghat were found to be quite dissimilar from each other. Distance matrix clearly signifies the very less difference among the sampled species proves that there is very less genetic diversity between them (Fig. 3). Molecular markers are very helpful in determining the gene flow and genetic differences within and between insect species which are essential for establishing a meaningful explanation for population structure and dynamics [45, 46, 47, 48]. Natural selection is another important component that is thought to be the driving force for population diversity. In this regard, molecular markers are employed to infer insect population phylogeny and biogeography, as well as to know the evolutionary processes and trajectories [49, 50, 51,52]. suggested that mitochondrial COI genes are well suited for determining genetic difference within species.

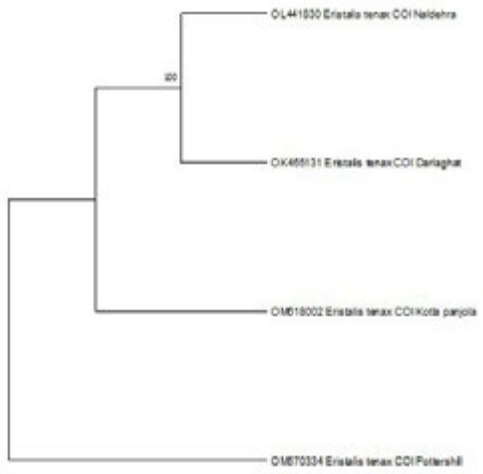

Figure 3: Phylogenetic tree of *Eristalis tenaxshowing* genetic relationships derived from COI sequences by using Neighbor-Joining method of MEGA X Software.

Results are in accordance with the recent findings of [53] also suggesting that honeybees are AT-biased and average A+T and C+G content of the findings in the ratio of 3:1 respectively. [54]who studied the genetic diversity in *Apiscerana* from 12 localities of Karnatka through COI gene of mitochondrial genome and also revealed the insignificant natural selection in the samples of *Apiscerana*.Similarly, [55] also studied the mitochondrial DNA diversity

of *Apiscerana* 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 *Apiscerana* were also strongly biased toward A and T with an average AT content of 75.6%. [56]alsoused mitochondrial DNA sequences to investigate the phylogeography of *Apiscerana* populations on Hainan Island and southern mainland China and discovered a significant phylogeographic structure within lineage. In 2019, Gaikwadstudied the phylogenetic variations in Apiscerana of North Western Ghats of Maharashtra, India. Western Himalayais one of the richest reservoirs of biological diversity in the world due to extreme variation in elevation and climatic conditions. Current study which is limited to the some of localities of Western Himalayas shows less genetic divergence. But a detailed sampling of *Eristalis tenax* across the Himachal Pradesh would provide interesting visions on the genetic diversity. Genetic diversity has been proved to be important for the fitness of species because harmful mutations can be counterbalanced by high levels of heterozygozity[57]. Environmental change is a significant and continual threat to the world's biodiversity. In order to survive, species must adapt to a constantly changing environment. As a result, in order to escape extinction, adaptation to environmental changes becomes critical (Hansen et al., 2012).However, around 40% of insect pollinators are threatened globally and their populations are dropping as a result of modern agricultures that allow the use of pesticides, as well as changes in global climate that have an impact on global food security (Khaikaew, 2016).

Intraspecific genetic variation is the most basic level of biodiversity since it provides the foundation for all evolutionary change[58]. Global climate change altered the intraspecific genetic diversity due to evolutionary consequences. It cause changes in the Individuals and communities phenotypic plasticity levels as they adjust to new environmental situations[59] and change also occur in the distribution of genetic variants when the ranges of populations and species change.In many circumstances, these alterations will limit genetic diversity in populations and species, leading to population viability and extinction in extreme cases.There is need of extensive sampling and further characterization of genetic diversitywith different mitochondrial genes to lessen the risk of extinction of species and ecosystems [60-66].

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Conflict of Interest

There is no conflicts of interest.

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