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Mitochondrial DNA reveals some ecological and evolutionary patterns that confer survival and reproduction values to various flies

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Abstract

Flies were collected from various garbage dumping sites. These were then morphologically identified and mitochondrial DNA (mtDNA) typing was performed for these. Then phylogenetic tree was attempted for deriving the inter relationships among these flies. Finally, these were analyzed for Phylogenetic niche conservation studies (PNC). Analysis revealed that all these species have retained certain niche related attributes all through the phylogeny (*viz.* these occupy same niche which is advantageous to the flies for oviposition and increase the probability of mate finding. Also, most of these flies have been found to obey Bergmann`s rule and larger body size connote some competitive advantage to these flies for better survival. Apart from this, some relevance of anautogeny in these flies also hint some relevance with fly borne diseases.

Keywords: mtDNA, PNC, flies, anautogeny

Introduction

Diptera being the second largest order comprises of 12-15% of total animal species. These exhibit varied ecological roles from detritivory to heamatophagy. Among these, the beckoning flies present exhilarant subject of study. Most of the flies (Scathophagidae, Calliphoridae, Muscidae) share a common niche as all of them nib and breed on dead decaying matter). Both molecular characterization and morphotaxonomy was performed for their identification. Before undertaking the ecological studies it was primemost to establish a phylogenetic relationship among these flies, for which mitochondrial DNA (mtDNA) was considered as the apt tool. Mitochondrial DNA (mtDNA) is more elucidative and easy to work with in comparison to nuclear DNA. Some of the valuable traits that make mtDNA indispensable are: relatively fast mutation rate; maternal inheritance, high copy number, circular molecule of small size ~16000bp; polyploid; intron free and shows almost no recombination. Also, phylogenetic relationship based on mtDNA has been quite successful (Bernasconi *et al*. 2000) [2]. Phylogenetic niche conservatism (PNC) is the property possessed by some of the species to conserve their niche-associated traits through the process of evolution. PNC is considered as a pattern, observed exclusively in some phylogenetically related species, as a consequence of some natural phenomenon. However, it is sometimes also considered as a process which itself generates some desirable characters (Crisp and Cook, 2012) [3]. This ecological pattern is of great value in research as these finally highlight some of the valuable mechanisms prevailing in nature which are valuable for the survival. In the present study, different flies (*Musca, Ophyra, Neomyia, Scathophaga* and calliphorids) have been studied for configuring some of the important phylogenetic niche associated patterns that have remained conserved generation after generation in the process of speciation**.** Analysis have shown that although, these species diverged from one another in the past (as are represented in a separate clade in phylogenetic tree), but, these have managed to retain their ancestral niche related traits *i.e.* all of them thrive in similar conditions (similar food and environmental preferences). This study indicates possibility of phylogenetic niche conservation pattern. Niche is the cluster of all the habitat characteristics and behaviours that allow a species to persist and produce offspring**.** Thus, when any characteristic pertaining to the niche or habitat of certain closely related species show some sort of similarity, it is considered as PNC. Phylogenetic tree was first of all constructed based on both UPGMA method to validate interrelationship between various species considered for PNC analysis. The phylogenetic analysis clearly described the relationships which provided the foundation for our analysis.

Methodology

The first step subsumed the collection of blow flies from different regions of North India (Himachal Pradesh, Punjab, Haryana and Uttrakhand), followed by sorting, pinning, labelling and identification of the collected specimens and finally preserving these identified samples in ethanol. The alcohol (Analytical CS Reagent, Changshu Yangyuan Chemical, China) preserved samples were washed in distilled water (Legency Remedies Private Limited, Baddi) to remove any foreign DNA or microbes. These samples were then dissected using sterilized surgical blade (Basil Orthopaedic Industries, Delhi). Only legs were dissected out and used for DNA isolation. Qiagen puregene DNA extraction kit (Gentra

Puregene Tissue Kit catalog number: 158667, MCS Biotech, Chandigarh) was used. A specific region of *COI* gene of mitochondrial DNA was chosen as the target for amplification. All polymerase chain reactions (PCR) were performed using Bio-rad T100 TM thermal cycler. The thermal cycler conditions were the following: initial denaturation at 98°C for 2 minutes followed by 40 cycles at 98°C for 30 seconds, annealing at 47.3°C for 30 seconds, elongation at 75°C for 30 seconds and final elongation at 75°C for 10 minutes. 50μL PCR cocktail constituted of Phusion DNA polymerase enzyme 1U/50μL reaction, 5X Buffer 10μL, 10pm dNTP, 50Mm $MgCl_2$ 1μL, 10pm primers 1uL each and MQ water (Thermo Fisher Scientific, India). This fragment of *CO1* gene was amplified using primers C1-J-2495 (5′ CAGCTACTTTAT GAGCTTTAGG) [Sperling, 1994] [6] and C1-N-2800 (5′ CATTTCAA GYTGTGTAAGCRTC) [Wells and Sperling 2001] [7]. Using ABI BigDye® Terminator v3.1 Cycle Sequencing reaction kit (Applied Biosystems, USA), the purified PCR amplicons were sequenced. Sequencing was performed on 3130 Genetic analyser Automated DNA sequencing machine. The softwares used for sequence analysis were Sequencing Analysis 5.1; Chromas Pro v3.1.

Result

Once sequences were procured, they were then submitted to the Genbank (accession numbers in table1). Further these sequences were considered for phylogenetic analysis. Then after, ecological aspects connoting niche conservation were studied.

Table 1: Sequences submitted to Genbank with their accession numbers.

Species	Accession number
Lucilia ampullaceal Villeneuve	KP325712
Ophyra leucostoma (Wiedemann)	KP862542
Scathophaga stercoraria (Linnaeus)	KP862544
Hemipyrellia ligurriens (Wiedemann)	KP325711
Hemipyrellia pulchra(Wiedemann)	KP325709
Chrysomya nigripes (Aubertin)	KP644240
Lucilia sericata (Meigen)	KP325713
Chrysomya albiceps (Wiedemann)	KP325710
Calliphora vicina Robineau-Desvoidy	KR080317

Phylogenetic analysis

The genotyping and phylogenetic methods used in this paper are widely and most commonly practiced.

UPGMA: The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length equal to 285.27110390 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence. The analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 525 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

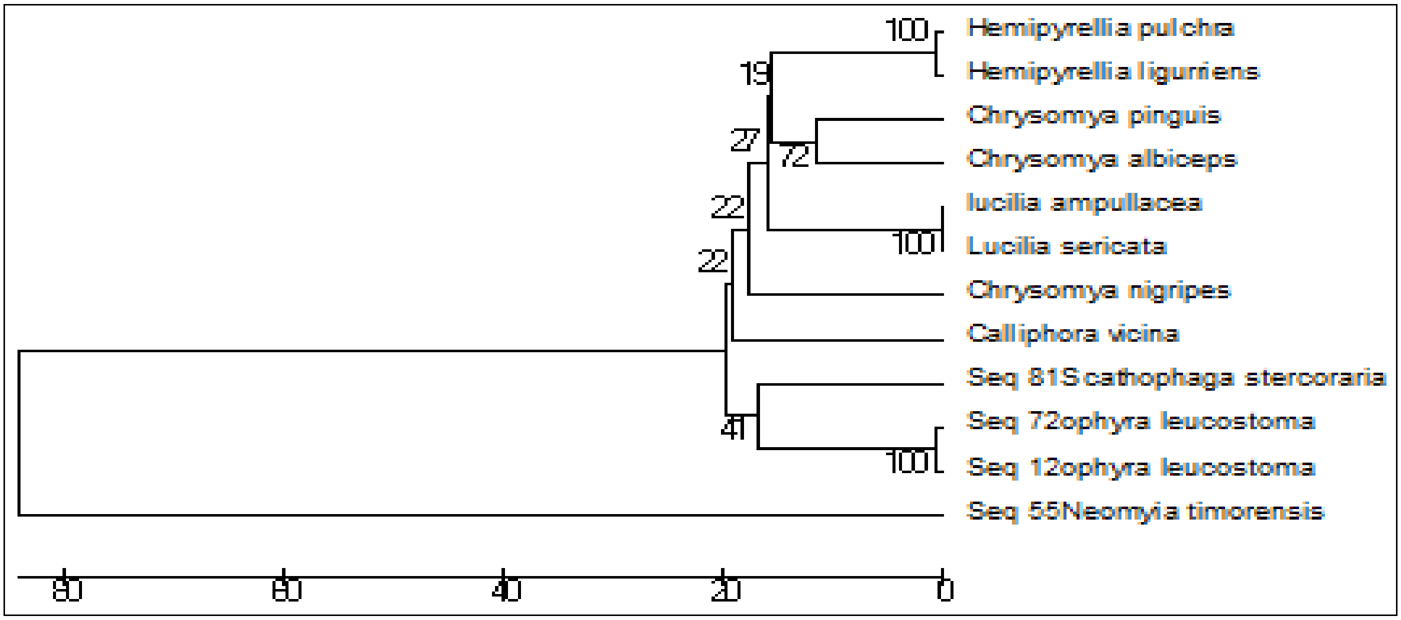


Fig 1: Phylogenetic tree showing evolutionary relationships among different taxa

Neighbour joining: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length equal to 279.70312500 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence. The analysis involved 12 nucleotide sequences. There were a total of 525 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

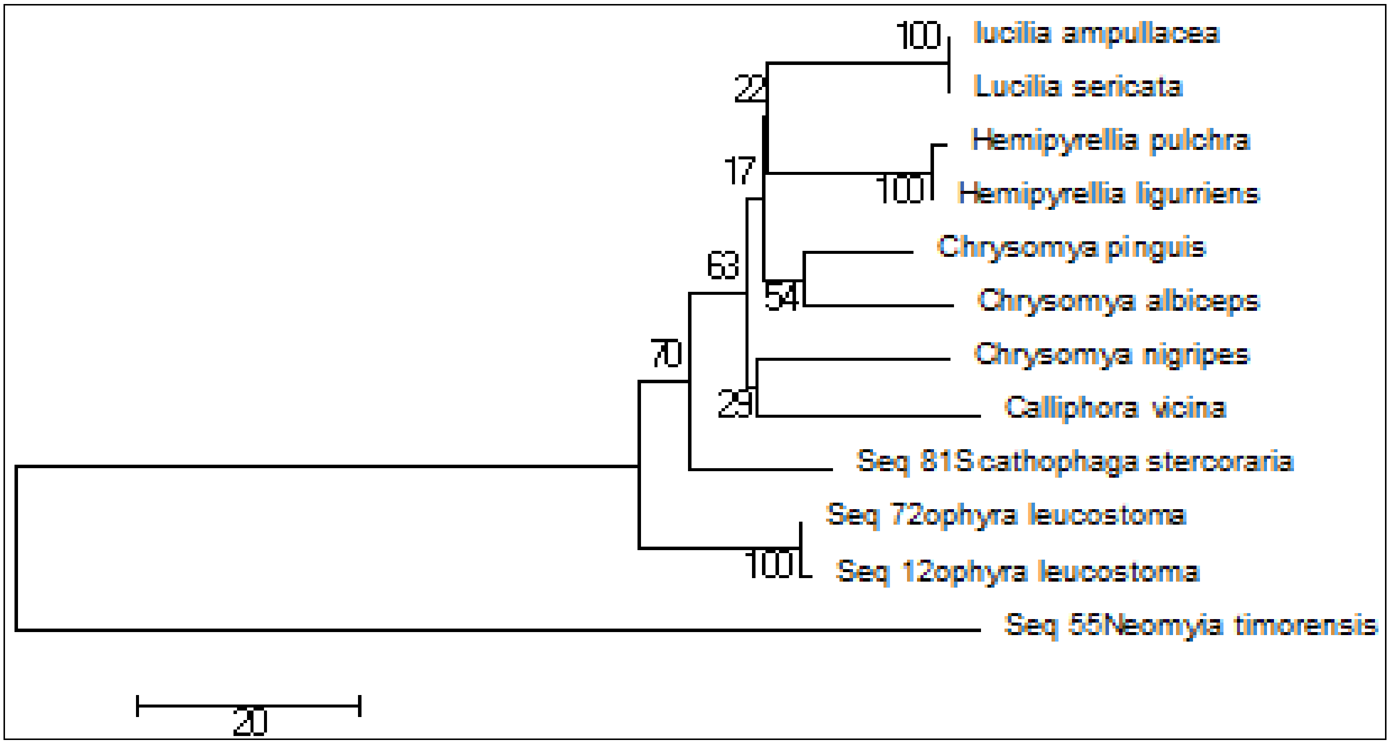
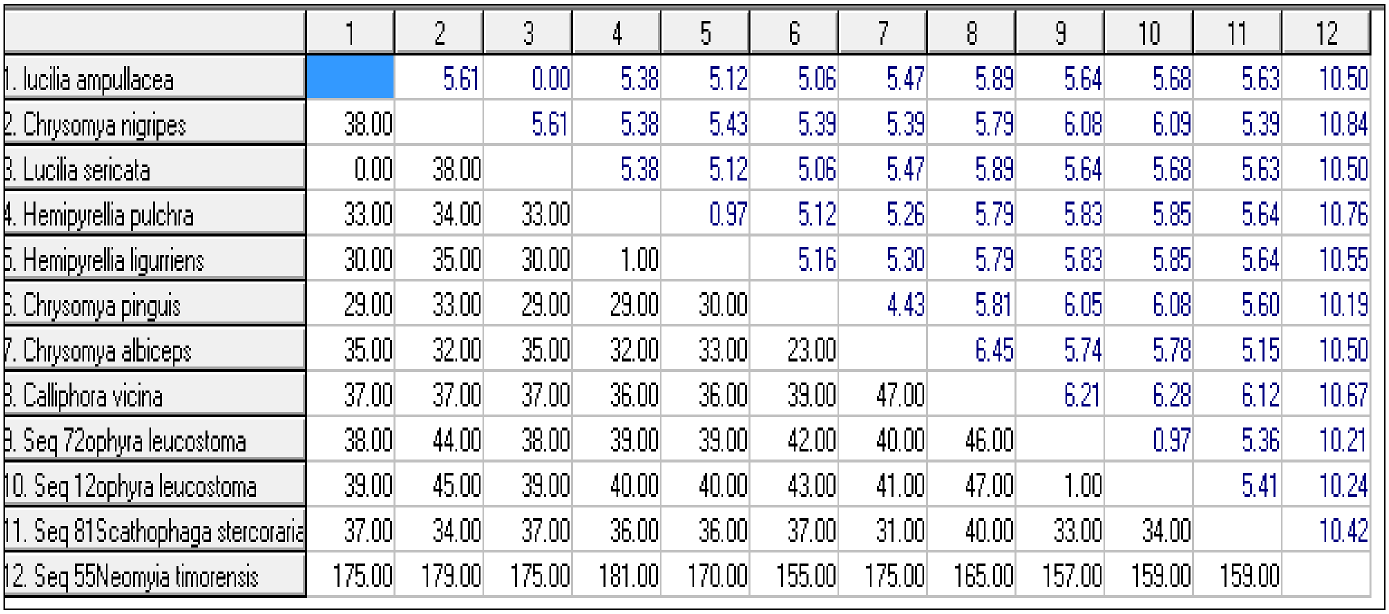


Fig 2: Phylogenetic tree showing evolutionary relationships among different taxa



	1	2	3	4	5	6	7	8	9	10	11	12
1. lucilia ampullacea	🟦	5.61	0.00	5.38	5.12	5.06	5.47	5.89	5.64	5.68	5.63	10.50
2. Chrysomya nigripes	38.00		5.61	5.38	5.43	5.39	5.39	5.79	6.08	6.09	5.39	10.84
3. Lucilia sericata	0.00	38.00		5.38	5.12	5.06	5.47	5.89	5.64	5.68	5.63	10.50
4. Hemipyrellia pulchra	33.00	34.00	33.00		0.97	5.12	5.26	5.79	5.83	5.85	5.64	10.76
5. Hemipyrellia ligurriens	30.00	35.00	30.00	1.00		5.16	5.30	5.79	5.83	5.85	5.64	10.55
6. Chrysomya pinguis	29.00	33.00	29.00	29.00	30.00		4.43	5.81	6.05	6.08	5.60	10.19
7. Chrysomya albiceps	35.00	32.00	35.00	32.00	33.00	23.00		6.45	5.74	5.78	5.15	10.50
8. Calliphora vicina	37.00	37.00	37.00	36.00	36.00	39.00	47.00		6.21	6.28	6.12	10.67
9. Seq 72ophyra leucostoma	38.00	44.00	38.00	39.00	39.00	42.00	40.00	46.00		0.97	5.36	10.21
10. Seq 12ophyra leucostoma	39.00	45.00	39.00	40.00	40.00	43.00	41.00	47.00	1.00		5.41	10.24
11. Seq 81Scathophaga stercoraria	37.00	34.00	37.00	36.00	36.00	37.00	31.00	40.00	33.00	34.00		10.42
12. Seq 55Neomyia timorensis	175.00	179.00	175.00	181.00	170.00	155.00	175.00	165.00	157.00	159.00	159.00	

Fig 3: Estimates of Evolutionary Divergence between Sequences

The analysis revealed the inter specific relationship between different species under consideration. Genera Hemipyrellia and Lucilia got rightly separated in the phylogenetic tree as a separate clade as both these belong to a common sub family Lucillinae. Similarly Chrysomyniae presented a separate branch of the tree but showing close relation with

Calliphorinae. The tree also correctly depicted the correct relation among different families namely Scathophagidae, Muscidae and Calliphoridae as all these furcated from each other as separate clade with sufficient variation as depicted in Figure 3. Also, Scathophagidae showed greater similarity with Calliphoridae than Muscidae.

Phylogenetic Niche Conservation

The ensuing points are some of the major attributes conferring to PNC.

- **All these flies rely on dung and decaying matter** Females of all these flies (*Musca*, *Ophyra*, Calliphorids) generally feed on organic matter but later gets attracted to dead bodies, wounded tissue, skin, hair, feathers. This may be because all of these flies have conserved this special physiological trait to process this kind of food matter. This is a common niche characteristic which seems to be advantageous to all these flies, so nature has conserved this niche related quality all through the phylogeny in some flies (Figure 4). Thus, both males and female flies are found to dwell on such matter [*e.g. Musca domestica* and *Ophyra* (Anderson, 2000) [1]. Thus, harbouring dead organisms provide both greater survival values and higher reproductive success. The following graph simply represents the number of a particular fly (genera) present at three different baits under considered under similar environmental conditions and same time.

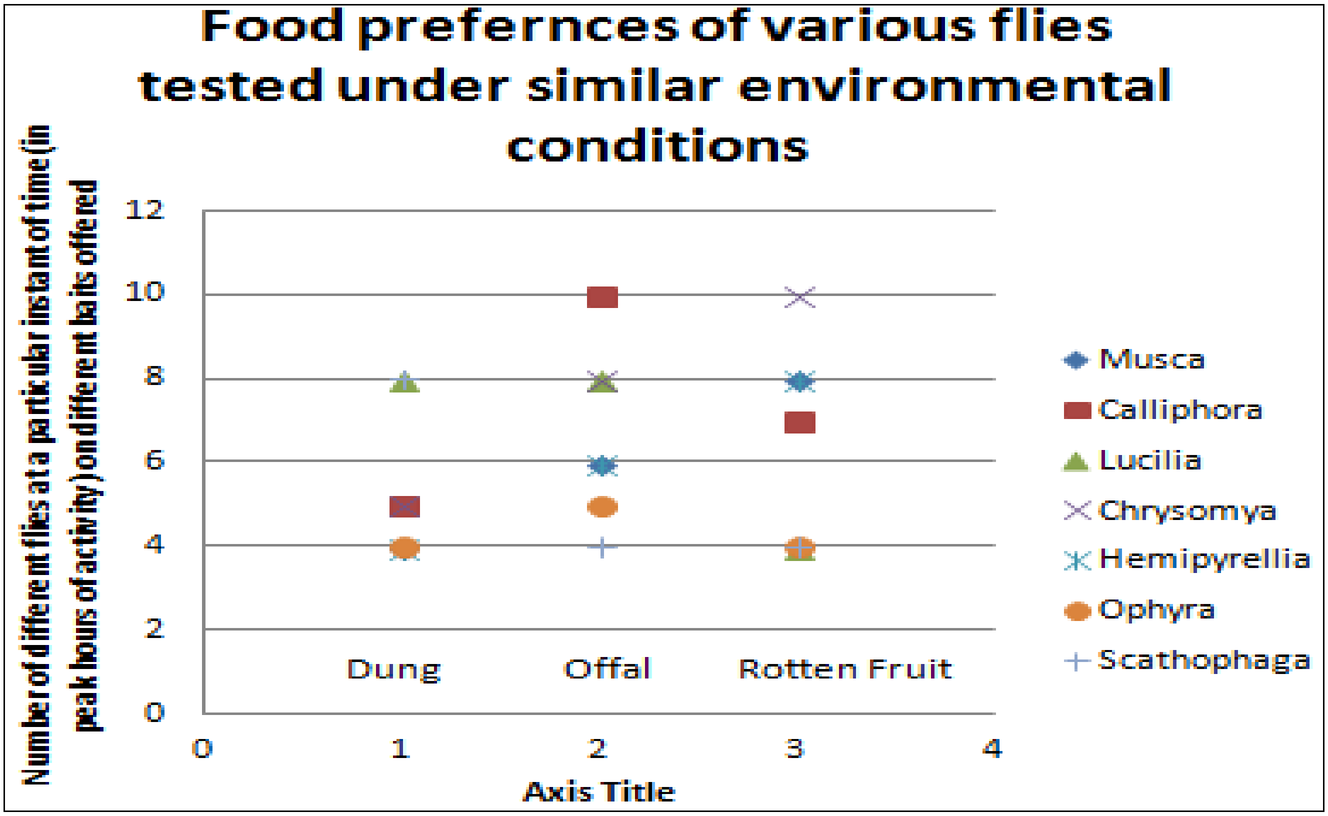


Fig 4: Food preferences of various flies

- **Anautogeny:** Adult females of most of these flies need blood meal for their completing their gonadotropic cycle. Also, it is advantageous for the survival of the vectors of various pathogens (these pathogens are tightly linked to adult females). It was also observed that, the insects feeding on vertebrate blood have higher fecundity rate than other insects.
- **Larger body size has a competitive advantage:** Larger body size is advantageous to the survival of flies as these would compete better for food and mate. These would better utilize the available resources and all this would finally lead to their propogation and survival. These would better contribute to the gene pool**.**
- **Phenotype varies with altitude:** In accordance with the Bergman`s rule, organisms at higher altitude tends to be larger in size. But, this observation generally does not apply to most of the organisms. But, in the present study, the author has found a certain trend of increase in size of the fly with increase in altitude. Except for altitude 500-1000mt. which generally shows a decrease in body size for most flies under consideration. This was done by calculating the Mean and Standard Deviation of lengths of particular genera of fly collected from a particular altitude and plotting a graph (Figure 5).

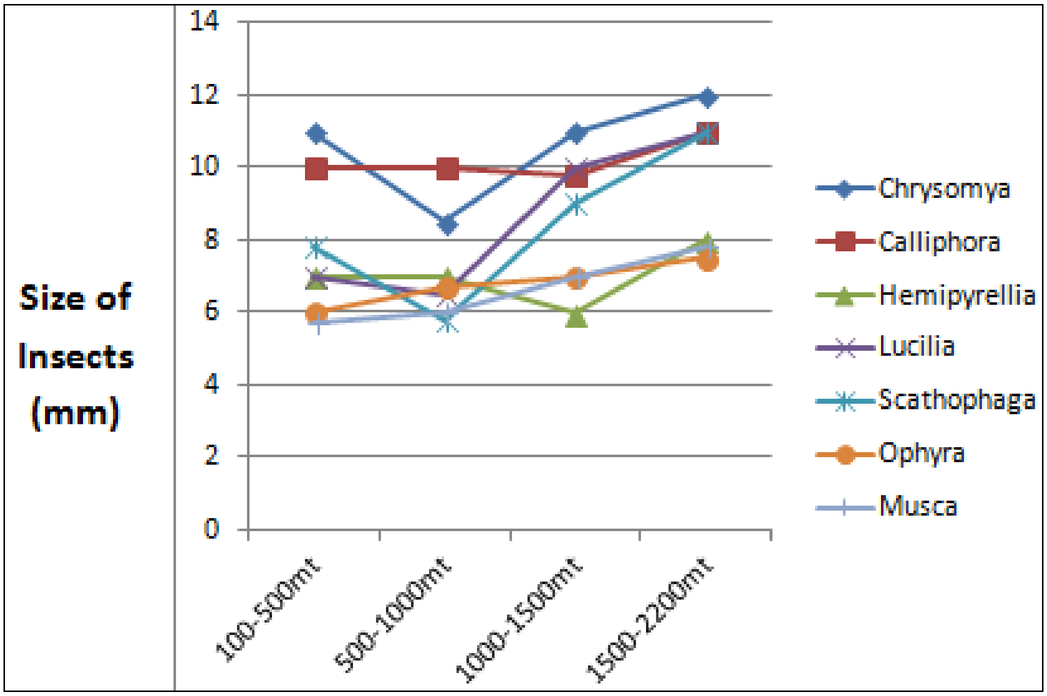


Fig 5: Variation in body size of various flies with altitude.

Discussion

It is very clear from the results that *COI* gene proves to be the best mitochondrial gene for identification purpose as well as for phylogenetic analysis. This gene has been very well studied at the biochemical level. Its size and structure is conserved in all aerobic organisms (Saraste, 1990) [5]. Also, it has been chosen as the perfect marker for the barcode of life. It is the largest protein coding gene, so alignment is easier due to the presence of amino acids. It is most suitable because highly conserved and variable regions are closely associated in the COI gene, so is perfect for tracing phylogeny.

Phylogenetic niche conservatism among the species considered in the present research is proved and depicted through various attributes. The author has attempted to relate some of the attributes (common to all these species) to PNC. Increase in body size with altitude could be explained by the fact that a larger body can accumulate more fat, which would provide some survival benefit to the fly in hours of scarcity of food. This trend was observed for most of the species considered for present study. However, further studies are due in this regard.All flies dwell on dead decaying matter and filth. This trait is beneficial; as it reduces the time the female flies have to spend away from their hosts and can oviposit more easily (Gubler, 1991) [4]. Also, it is lucrative to the larvae, for, they find a food rich environment upon hatching, thus insuring their survival. This easy availability of females on vertebrate hosts is advantageous to the males as it leads to greater mating success. Presence of sugars, fats on such corpses along with an easy access to their mates has made it convenient through evolution to bring about specific physiological changes in these flies. Anautogeny is known for all these flies, which seems to explain some significant relationship with pathogens of various diseases, which have with time learned to adjust to the life cycle of these flies. These findings would be very useful in studying various aspects of fly borne disease.

All these attributes (chosen for analysis) do have some relation with the niche and show some specific conservation patter in all these species. The author feels that any process that is selected by nature (generations after generations) must hold some lucrative role in the survival and reproduction of these species. Any trait, that increases the survival value of a species or enhance the reproductive quality of the individual is usually selected by nature and eliminated if it is detrimental to the survival of organism. The closely related species tend to occupy similar niches and distant relatives possess different characteristics to adjust in the similar habitat. Thus, resource partitioning is a substitute to their survival.

Conclusion

The author wants to convey that most of the observations and interpretations drawn from present study were very encouraging. These connote at some new prospects in research. There were some entirely fresh findings which would aid in unfolding some other latent phenomenon conferring to insects and molecular studies as mentioned below:

1. *COI* gene has been found to be an apt tool for molecular characterization of dipteran flies. Thus, present research further buttresses the reliability of *COI* gene for DNA barcoding.
2. Also, *COI* gene has rightly derived the phylogenetic interrelationship between the flies (*Musca, Ophyra, Neomyia, Scathophaga* and various calliphorids). Thus also implies its importance as a phylogenetic tool.
3. Scathophagidae have been found to be a closer relative of calliphoridae than Muscidae.
4. All these flies have been found to share same ecological niche (feeding and breeding in similar environment) which provides them better chance of mate finding and

better survival fitness value to larvae.

5. Most of the flies at higher altitudes have been found to have larger body size which provides for better survival in times of scarcity of food and better heat insulation at low temperature (as larger body size implies to more fat storage).
6. Also all these flies have been found to be anautogenic which hints at higher fecundity. Thus all these factor connote at Phylogenetic Niche Conservation.

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Disclosure

The authors are not conversant of any memberships, financial holdings, affiliations that could raise a conflict of interest.

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