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Short Communication

Mitochondrial CR and nuclear ITS2 regions analysis in flesh flies

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Abstract

Flies (flesh flies) of the family Sarcophagidae are found all over the world and are considered to be of great forensic and medical importance. These are difficult to identify by morphological characters, therefore, molecular marker technology based methods prove to be more effective and are used as an alternative method over morphology based identification. The use of molecular marker technology for exploring genetic relationship/identity or for forensic studies is most effective and precise method also. In this paper an attempt has been made to analyse mitochondrial CR and nuclear ITS2 region among flesh flies to draw genetic closeness.

Keywords: Flesh flies, CR, ITS2, Nuclear region, molecular marker.

Introduction

Members of the family Sarcophagidae are commonly known as flesh flies because the larvae of these flies feeds on flesh causing myiasis, these flies are medically important because these are responsile for the transmission of pathogenic bacteria and viruses[1-3]. These are also used in the determination of PMI therefore these contribute a great deal in forensic studies also[4-6]. These flies are distributed globally and are of synanthropic nature. Because of their great medical and forensic importance various DNA based studies are going on all over the world for accurate identification process[7-10]. However, molecular markers involving mitochondrial regions are also used in these flies for determination of genetic closeness or for phylogenetic relationship studies[10-11].

In the present study, mitochondrial Control Region (CR) and nuclear Internal Transcribed Spacer 2 (ITS 2) regions are sequenced to draw genetic relationship (closeness) among five species of flesh flies i.e. *Sarcophaga albiceps*, *S. dux*, *S. ruficornis*, *S. argyrostoma* and *S. knabi*.

Materials and methods

For DNA extraction method, Maniatis *et al.* process has been followed[12]. For amplification of mitochondrial Control Region (CR) the primer pair used are 5' ATTTACCCTATCAA GGTAA 3' and 5' AATCCAGTTAAGAATATCAT 3' and for nuclear Internal Transcribed Spacer 2 (ITS2) region the primer pairs were 5' TGCTTGGACTACATATG GTTGA 3' and 5' GTAGTCCCATATGAGTTGAGGTT 3'. The 25µl reaction mixture for amplification of both the regions contain 10X PCR buffer, 0.25 mM dNTP, each primer (10 picomole), 1.5U Taq DNA polymerase, extracted DNA (30ng) and milli Q water and the amplification profile for CR have initial denaturation of 4 minute at 94°C (1 cycle), 34 cycle of denaturation for 1 minute at 94°C temperature, annealing of 1 minute at 45°C temperature, extention of 2 minute at 60°C temperature and final extention for 10 minute at 72°C temperature (1 cycle) while for ITS 2 region initial denaturation of 5 minute at 94°C temperature (1 cycle), 30 cycle of denaturation for 1 minute at 94°C temperature, annealing of 1 minute at 42°C temperature, extention of 2 minute at 72°C temperature and final extention for 7 minute at 72°C temperature (1 cycle). From Genei, sequencing was performed for both the regions and sequenced regions get accession numbers from Gen Bank (Table-1). For alignment of CR and ITS2 regions a computer software known as Cluxtal X was involved while for calculating different statistical values another computer software known as MEGA 4 was used[13-14].

Results and discussion

This study is the first study involving mitochondrial CR and nuclear ITS2 regions for flesh flies belonging to genus *Sarcophaga* from India. The length of the CR amplicon ranges from 436 to 456 bp. The gene sequence of CR amplicon reveals 127 sites which are variable and 58 sites which are parsimony informative, however, average A, G, C, T nucleotide ratio was 49: 4: 5: 42, respectively and 0.18 value was observed for transition bias. However, for ITS2 amplicon the length varies from 385 to 397 bp with 175 sites for variable value and 67 sites for parsimony informative value. Average A, G, C, T base ratio was 38: 11: 13: 38 while 0.37 value was observed for transition bias. Pair wise nucleotide difference ranges from 0.058 to 0.306 for CR (average value 0.184) and 0.321 to 0.639 for ITS2 (average value 0.510) amplicons, respectively (Table-2). Neighbor Joining dendogram have been used for phylogenetic relationship inferred from CR and ITS 2 regions.

Table 1 Accession numbers for CR and ITS 2 regions

Species	Region and length in base pair	Accession number
S. dux	CR, 437	FJ946633
S. knabi	CR, 438	FJ946635
S. ruficornis	CR, 443	FJ946639
S. argyrostoma	CR, 456	FJ946631
S. albiceps	CR, 436	FJ946630
S. dux	ITS 2, 397	HM216415
S. knabi	ITS 2, 391	HM216416
S. ruficornis	ITS 2, 385	HM216417
S. argyrostoma	ITS 2, 387	HM216413
S. albiceps	ITS 2, 389	HM216412

Table-2: Pair wise nucleotide difference for CR (in italics) and ITS2 amplicons.

	S. argyrostoma	*S. ruficornis*	*S. knabi*	*S. albiceps*	*S. dux*
S. argyrostoma	-	0.096	0.148	0.154	0.147
S. ruficornis	0.090	-	0.129	0.137	0.140
S. knabi	0.161	0.105	-	0.113	0.086
S. albiceps	0.306	0.246	0.166	-	0.071
S. dux	0.303	0.240	0.163	0.058	-

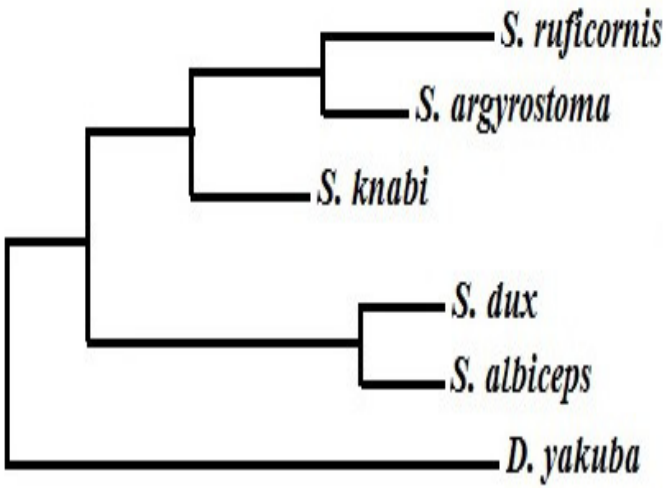

Figure-1: Neigbor joining dendogram for CR.

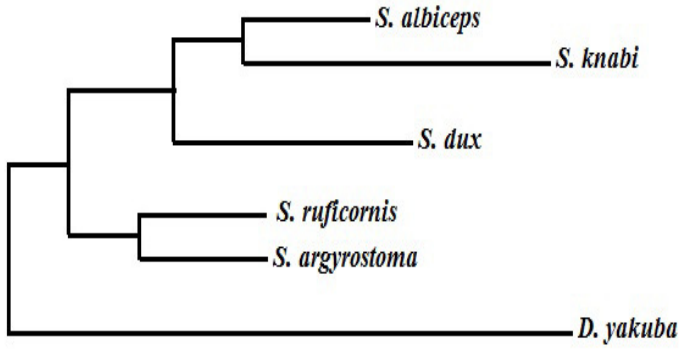

Figure-2: Neigbor joining dendogram for ITS2 region.

Mitochondrial and nuclear gene loci were widely used among flesh flies for genetic closeness analysis and genetic identity determination[7-11,15-16]. Heteroplasmy in length was observed for both CR and ITS2 amplicon which is due to tandem repetition and copy number variation in nucleotides[15,17-18]. The value of transition bias in CR and ITS 2 (non coding regions) is low as compared to other coding regions studied[8,11]. High transition bias values are observed in coding regions because in coding regions non synonymous transversion is selected because transversion results in greater biochemical difference in protein obtained from coding region[19]. The A, T nucleotide content of CR and ITS2 region in five sarcophagid flies was found to be very high, in CR this may be because of AT favoured mutational changes exerted early on the radiation of insects[17]. The AT bias of ITS2 region in *Drosophila* has been assumed to be the result of rDNA clusters which are located in heterochromatin[15,20]. Studies involving bright fluorescence method also reveals high AT content in sarcophagid flies[21-22]. Pair wise nucleotide difference value for CR amplicons reveals high sequence similarities which may be due to recent divergence among these flesh flies[23-24]. However, a high sequence difference value for ITS2 region may be indicative of high mutation rate and faster evolution of this region as compared to CR[25].

For phylogenetic analysis Neighbor Joining method has been used which groups *S. ruficornis* and *S. argyrostoma* in one cluster while *S. dux* and *S. albiceps* in another cluster for both the amplicons, however, the position of *S. knabi* changes. Since, different regions of the genome evolve at different rate, certain discrepancies are bound to appear. However, to resolve the evolutionary picture more clear comparision of present data with other regions is necessary because informative power of the combined regions increases[25-26].

Conclusion

It may, however, be concluded from the foregoing, that sarcophagids have undergone very little change as revealed by comparision of different molecular markers. The author is aware that much more evidence is required, especially at the level of DNA based studies, to draw any correlation between rate of speciation and evolutionary change.

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