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Random amplified polymorphic DNA based characterization of flesh flies

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Abstract
Flesh flies are medically important flies as they cause myiasis in livestocks. At an early stage of development these flies are morphologically identical. Therefore, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique was used to explore its importance as genetic marker for identification process. From the twelve primers used for RAPD-PCR method, different species specific bands were obtained, which can be further utilized for identification purpose and the data obtained support the RAPD-PCR methods ability to distinguish between two flies of the genus *Sarcophaga* i.e. *S. albiceps* and *S. knabi*. The mean heterozygosity observed in *S. knabi* was 0.150 and in *S. albiceps* it was 0.114, suggesting that there exists a low genetic variation in these species.

Keywords: flesh flies, RAPD-PCR, myiasis, genetic marker, primers

Introduction
The family Sarcophagidae includes flesh flies which are distributed all over the world and are synanthropic flies also [1]. Some members belonging to this family cause myiasis as the larvae feeds on flesh [2]. At an early stage of development these flies are morphologically identical [3, 4]. Now days, DNA based techniques are commonly used to genetically characterize different members of Dipteran families [5-8]. Among all other DNA based methods RAPD-PCR is more frequently used as it allows DNA polymorphism within short duration of time. The bands produced by RAPD markers are amplified random DNA segments from arbitrary primers. This method has been utilized for different genetic and systematic studies. One of the most advantageous aspect of this method is the utilization of little DNA concentration [9, 10].
For identification, grouping and phylogenetic conclusions the morphology of male genitalia, especially the development of ventral sclerotization has been the only character in these species which offers good morphological evidence [11, 12]. However, a meaningful correlation between evolutionary change at morphological and molecular level could only be carried out by studying molecular data [11, 12]. Therefore, in the present study, twelve decamer primers were used to analyze different patterns of bands between *Sarcophaga knabi* and *Sarcophaga albiceps* with a view to establish RAPD marker as an important method to facilitate the identification of these species. Genetic closeness i.e. average heterozygosity was also calculated between these species so that the similarity between analyzed *Sarcophaga* species was also unravelled.

Materials and Methods
Genomic DNA was extracted by homogenizing individuals in homogenizing buffer (50mM EDTA, 0.1M Tris, 0.2M sucrose and 0.5% SDS (pH-8.0)). The extracted products were RNase digested with an incubation of 30 min. at 37 ºC after which DNA was phenol / chloroform extracted, ethanol precipitated and resuspended in 100 µl Tris: EDTA buffer (pH- 8.0). Twelve decamer primers were used for amplification of the genomic DNA of both the species (Table 1). For Polymerase chain reaction amplifications, gel visualization and bands interpretation the method adopted by Bajpai and Tewari [13] was followed. The DNA markers used in the gel for fragment size determination was low range DNA ruler (100-3000 bp). The gels were photographed with Biovis Gel Documentation System and analyzed with Biovis V4 1D Gel. The presence/absence of each bands from 30 individuals of both the species were used as a data matrix to analyze average heterozygosity with Tools for Population Genetic Analysis (TFPGA) Software [14].

Results and Discussion

The decamer primer used, amplicons size, heterozygosity and total numbers of amplified fragments are shown in table 1. The minimum number of scored fragment in *S. knabi* was 1 (590 bp) while in *S. albiceps* it was 0 with primer number 3 i.e. GTAGACCCGT. However, the maximum number of fragment scored in *S. albiceps* was 6 with primer number 9 and 12, while, in *S. knabi* the maximum number of scored fragment was also 6 with primer number 5. The smallest size fragment scored was of 181bp with primer number 9 while the largest fragment of size 1850bp was scored with primer number 6. The minimum and maximum heterozygosity observed in *S. albiceps* were 0 and 0.321 with primer number 11 and 10, respectively. In *S. knabi* minimum heterozygosity observed was 0.043 with primer number 7 and maximum heterozygosity was 0.464 with primer number 3. Amplification of twelve primers produced a total of 77 bands between the two species which ranges from 181 bp to 1850 bp. However, from 77 bands 36 fragments were scorable in *S. knabi* which ranges from 220 bp to 1850 bp and out of 36 scorable fragments 4 (9%) fragments were monomorphic. In *S. albiceps* 41 fragments were counted which ranges from 181 bp to 1486 bp and out of 41 fragments amplified 12 (29.26%) fragments were monomorphic. The decamer primer 5' TCGCATCCCT 3' of the present study was able to generate specific banding pattern considered to be ideal for species identification and also the pattern was monomorphic i.e. present in 100 % individuals in *S. albiceps* while rest of the primers were unable to generate a pattern which is monomorphic (Fig. 1 (a)). The mean heterozygosity in *S. knabi* and *S. albiceps* were 0.150 and 0.114, respectively, suggesting a low genetic variation in these species. A comparison of other molecular data also reveals very close relationship among members belonging to the genus *Sarcophaga* [5, 15-17]. However, it is imperative to use additional DNA based methods in these flies for genetic characterization so that more specific information can be drawn for clarifying genetic relationship. Figs. 1 a and b represents the amplification pattern of two species with primer 5' TCGCATCCCT 3'

Table 1: The primers used, number of fragments amplified, range of size of amplified fragments and average heterozygosity in two species:

S. No.	Primers used (5'-3')	Number of amplified fragments in		Range of size of amplified fragments (in base pairs)
		S. albiceps	*S. knabi*	
1	AGGGCGTAAG	2 (0.088)	2 (0.087)	237-630
2	GTCCCGACGA	2(0.284)	3 [1] (0.097)	408-1289
3	GTAGACCCGT	-	1 (0.464)	590
4	GGTGCTCCGT	4 [3] (0.033)	3 [1] (0.105)	390-1480
5	ACGGATCCTG	4 (0.160)	6 (0.214)	254-1426
6	CCTGATCACC	2 [1] (0.045)	2 [1] (0.049)	436-1850
7	GGTGATCAGG	2 [1] (0.037)	2 [1] (0.043)	415-1070
8	CCGAATTCCC	4 [2] (0.037)	2 (0.059)	231-1000
9	CTCAGTGTCC	6 (0.220)	3 (0.115)	181-1219
10	CTGGACGTCA	4 (0.321)	5 (0.283)	431-1486
11	TCGCATCCCT	5 [5] (0)	4 (0.206)	242-1108
12	ACGGTACCAG	6 (0.151)	3(0.083)	200-1000

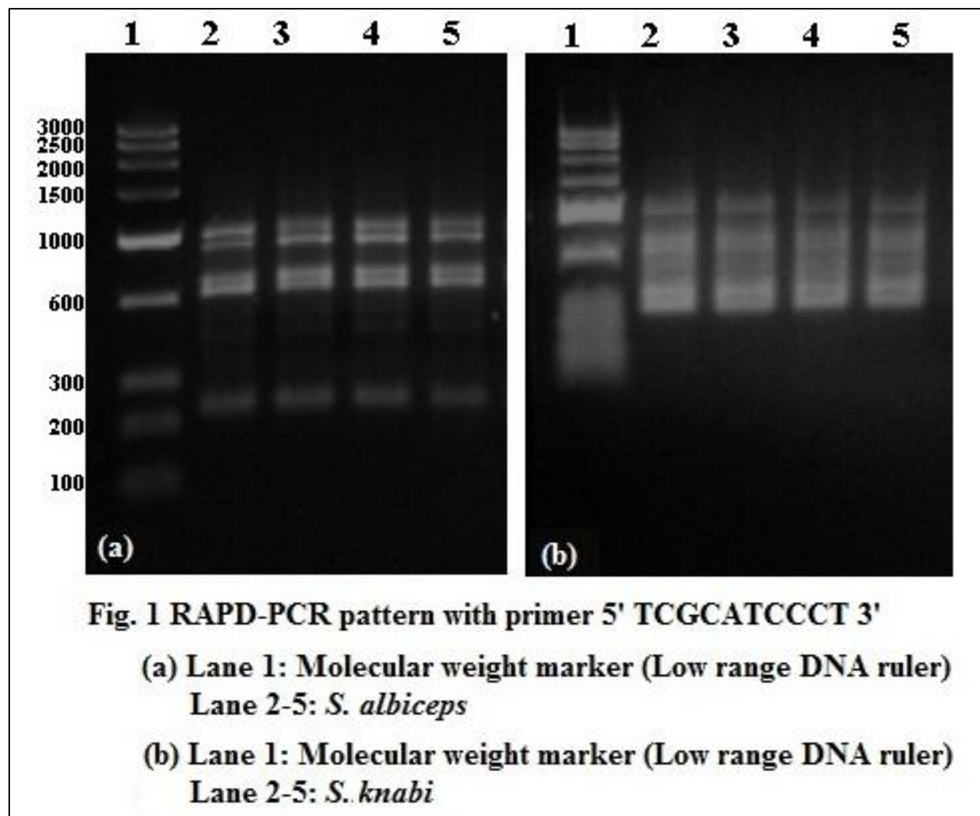

Fig. 1 RAPD-PCR pattern with primer 5' TCGCATCCCT 3'

(a) Lane 1: Molecular weight marker (Low range DNA ruler)
Lane 2-5: *S. albiceps*

(b) Lane 1: Molecular weight marker (Low range DNA ruler)
Lane 2-5: *S. knabi*

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References

1. Zumpt F. Myiasis in man and animals in the old world. Butterworth, London, 1965.
2. Tewari RR, Thakur S Acid phosphatase isozymes in five species of sarcophagid flies. Nat. Acad. Sci. Letters, 1991; 14:351-354.
3. Carvalho CJB, Mello-Patiu CA. Key to the adults of the most common forensic species of Diptera in South America. Rev. Bras. Entomol, 2008; 52:390-406.
4. Giroux M, Pape T, Wheeler TA. Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. Zool. J Linn. Soc., 2010; 158: 740-778.
5. Zehner R, Amendt J, Schutt S, Sauer J, Krettek R, Povolny D. Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). Int. J. Legal Med., 2004; 118:245-247.
6. Guo YD, Cai JF, Xiong F, Wang HJ. The utility of mitochondrial DNA fragments for genetic identification of forensically important sarccophagid flies (Diptera: Sarcophagidae) in China. Trop. Biomed, 2012; 29:51-60.
7. Amorim JA, Souza CM, Thyssen PJ. Molecular characterization of *Peckia (Patonella) intermutans* (Walker 1861) (Diptera: Sarcophagidae) based on the partial sequence of the mitochondrial cytochrome oxidase I gene. J Forensics Res. 2014; 5:3.
8. Napoleao KS, Mello-Patiu CA, Oliveira-Costa J, Takiya DM, Silva R, Moura-Neto RS. DNA-based identification of forensically important species of Sarcophagidae (Insecta: Diptera) from Rio de Janeiro, Brazil. Genet. Mol. Res., 2016; 15:1-7.
9. Skoda SK, Pornkulwat S, Foster JE. Random amplified polymorphic DNA markers for discriminating *Cochliomyia hominivorax* from *C. macellaria* (Diptera: Calliphoridae). Bull. Entomol. Res., 2002; 92:89-96.
10. Jain SK, Neekhra B, Pandey D, Jain K. RAPD marker system in insect study: A review. Indian J. Biotech., 2010; 9:7-12.
11. Carvalho CJB, Mello-Patiu CA. Key to the adults of the most common forensic species of Diptera in South America. Rev. Bras. Entomol, 2008; 52:390-406.
12. Giroux M, Pape T, Wheeler TA. Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. Zool. J Linn. Soc., 2010; 158:740-778.
13. Bajpai N, Tewari RR. Genetic characterization of sarcophagid flies by Random Amplified Polymorphic DNA-Polymerase chain reaction (RAPD-PCR). Nat. Acad. Sci. Lett., 2010; 33:103-106.
14. Miller MN. Tools For Population Genetic Analysis (TFPGA) 1997.(http://bioweb.usu.edu/mpmbio/)
15. Wells JD, Pape T, Sperling FAH. DNA based identification and molecular systematics of forensically important Sarcophagidae (Diptera). J Forensic Sci. 2001; 46:1098-1102.
16. Song ZK, Wang XZ, Liang GQ. Species identification of some common necrophagous flies in Guangdong province, southern China based on the rDNA internal transcribed spacer 2 (ITS2). Forensic Sci. Int., 2008; 175: 17-22.
17. Bajpai N, Tewari RR. Molecular characterization of the genus *Sarcophaga* using PCR-RFLP markers. Nat. Acad. Sci. Lett., 2010; 33:47-51.