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Genetic Relationship between the Muscids Using RAPD-PCR as marker

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Abstract

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) markers have become the most common yardsticks to study genetic relatedness and to resolve intra and inter-specific relationship in many insect groups. In the present study, RAPD-PCR has been used to infer genetic relationship between the two muscid species i.e., housefly Musca domestica (L.) and pepper fruit fly Atherigona orientalis. Genomic DNA from the two species were extracted and PCR amplified using twenty arbitrary decamer primers which produced 127 reproducible and scorable bands ranging from 121bp to 2568bp. Genetic identity between the two species was calculated using Tools for population genetic analysis(TFPGA) software. High values of Genetic identity (0.680) display close relationship between the two species.

Keywords: RAPD-PCR, Muscidae, Housefly, Pepper fruit fly, Genetic relationship.

Introduction

The housefly (*Musca domestica* L.) is a highly mobile cosmopolitan pest of medical and veterinary importance[1]. It has been established that housefly is a carrier of several pathogens causing serious and prevalent diseases such as salmonellosis, poliomyelitis, typhoid fever, cholera, infantile diarrhea and amoebic dysentery[2-4]. *Atherigona orientalis,* commonly called as pepper fruit fly is an economically important fly, found in most tropical and subtropical areas of the world and is usually considered a secondary pest or "trash fly". However, it can sometimes be a primary pest of certain agricultural crops, most notably plants in the family Solanaceae.

Random Amplified Polymorphic DNA is a dominant expression marker for which no prior information about genomic organization is required[5]. The method is cost effective and uses ten base primers of arbitrary sequence which amplify numerous regions of the genome. The amplicons thus generated reveal abundant genetic information by comparing the genomic fingerprints and can be used successfully for identification and assessing genetic relationships in dipterans of medical, veterinary and economic importance[6- 13].

RAPD-PCR has been used to infer genetic relationship between two muscids namely, housefly *Musca domestica* (L.) and pepper fruit fly *Atherigona orientalis* (Schiner) in the present study.

Material and Methods

Sweep nets were used to collect houseflies whereas the pepper fruit flies were collected from infested brinjal fruits. Flies were identified and reared in cages at 26±1°C. Genomic DNA was isolated, resuspended in EDTA and quantified using UV-Vis Spectrophotometer (Elico)[11]. The purity of DNA was determined by analyzing the ratio of the optical densities at 260 nm and 280 nm. The quality of DNA was assessed by running 20µl of the extracted DNA sample on 1% agarose gel. Extracted DNA was stored at -20°C for further use.

The sequences of the primers (procured form Bangalore Genei) used in present study have been presented in table-1[6,14]. The reaction mixture was prepared at 4°C and 20µl of which comprised of 2µl of 10X assay buffer (provided by the manufacturer), 1.5µl 10 mM dNTP, 1µl of primer (containing 30 picomoles of primer), 0.5µl of Taq DNA polymerase, 30ng of genomic DNA and 15µl milli Q water. Amplifications were performed in BIORAD thermal cycler. The amplification profiles of all the primers are presented in table-1.

In order to detect any DNA contamination a negative control reaction was carried out for each primer sans the template DNA. For each individual the amplification reactions were repeated twice for each primer to determine consistency and reproducibility of the bands. The amplicons were loaded parallel with the low range DNA ladder (100 bp - 3000 bp) (Bangalore Genei) and electrophoresed on 1.5% agarose gel[15].

Gels were photographed under Biovis Gel Documentation System, band detection and their molecular weight interpretation was determined using Biovis V4 1D gel analysis software taking low range DNA ruler as molecular weight marker. On the basis of presence or absence of bands in 30 individuals of each species, a data matrix was prepared. Only those bands which were present in more than 70% individuals were considered for

calculation. Smears were not taken into consideration. Since RAPD is dominant expression marker hence a dominant allele at a particular locus is denoted by the presence of a band whereas the absence of a band is a manifestation of a homozygous recessive allele at that locus. Tools for Population Genetic Analysis (TFPGA) Software[16] were used to calculate of Nei's (1972) genetic identity[17] average heterozygosity and clustering of the species.

Results and Discussion

Both the species revealed a total of 127 bands ranging from 121 bp to 2568 bp out of which 113 fragments were amplified in *M. domestica* and 95 in *A. orientalis*. 14 and 25 fragment was found to be monomorphic in *M. domestica* and *A. orientalis* respectively (table-2). The bands which were present in all the individuals have been considered to be monomorphic. Figures-1, 2 and 3 portray amplification patterns produced by primer 9, 17 and 18 in both the species.

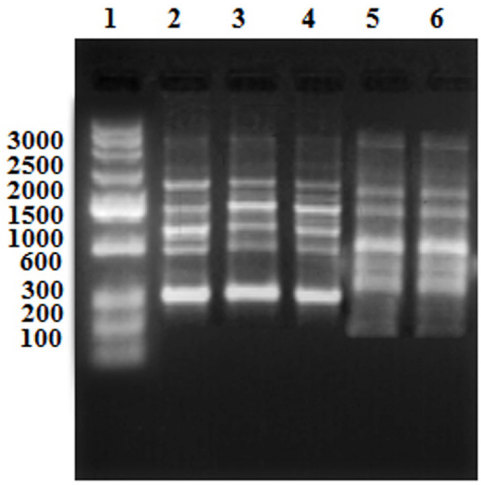

Figure-1

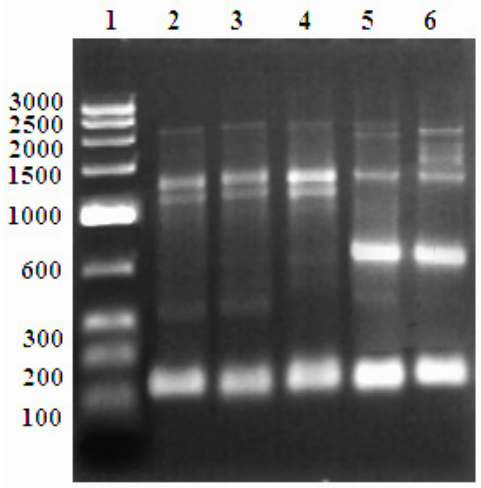

Figure-2

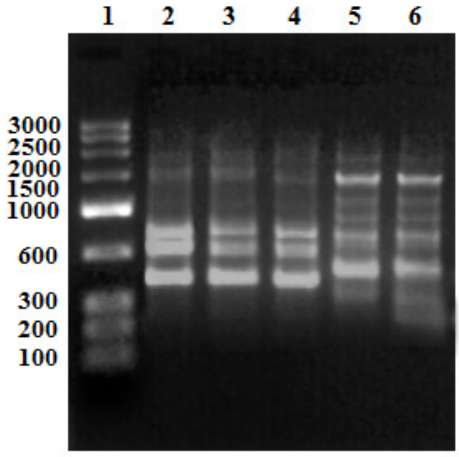

Figure-3

Fig. 1, 2 and 3 represent RAPD amplification patterns in the two muscid species as revealed by primer 9, 17 and 18

Lane 1: Low range DNA ruler (100-3000 bp), Lane 2-4: *M. domestica*, Lane 5-6: *A. orientalis*

Discussion: RAPD-PCR is an efficient method for analyzing genetic polymorphisms among diverse group of organisms[6-8,11,12,18]. The technique utilizes random decamer sequences as a single primer that anneals and primes at multiple locations throughout the genome of an organism. The patterns of amplicons produced are characteristic of the template DNA[5,20]. The presence and absence of a specific PCR product is diagnostic for the primer binding sites on genomic DNA, therefore, can serve as useful molecular markers for genetic characterization as well as assessment of genetic relationships[21]. The advantages of RAPD-PCR include its cost effectiveness, small DNA sample requirements, no requirement of prior knowledge of the sequence of DNA and the ability to identify hundreds of new markers in a short time[22].

The high average heterozygosity values obtained, 0.197 in *M. domestica* and 0.139 in *A. orientalis,* are somewhat greater than that of other muscid species viz., *M. domestica* (0.163)[23], *Stomoxys calcitrans* (0.070)[24] and *Haematobia irritans* (0.131)[25]. This may be ascribed to the fact that RAPD loci have higher mutation rates, hence are more polymorphic than that of other markers [19]. Different regions in the genome evolve at different rates and single-copy genes have relatively low substitution rates because some alteration in a coding sequence may cause a severe effect on the coded protein product. However, changes in repetitive DNA apparently do not result in these consequences and thus the polymorphism in these regions is greater than coding regions[26].

Greater population density of *M. domestica* is a reason behind the higher heterogeneity of the fly, as a species distributed over a large area is subjected to greater variety of environmental conditions, thus would be genetically more heterogeneous as compared to the species spread over a restricted area[27]. Thus

greater heterogeneity in housefly makes it able to survive and successfully cope up with environmental stresses[28].

The intra-specific genetic identity values in both the species are higher than 0.855, as a narrow genetic pool is represented by the individuals of the same species[29]. High genetic identity values between the two muscid species (0.680) may be coherent to the fact that *M. domestica* and *A. orientalis* belong to closely related subfamilies of Muscidae i.e., Musciinae and Phaoniinae, respectively[30].

Conclusion

The present study indicates the utility of RAPD-PCR technique to unravel genetic relationships among muscids. It may, however, be concluded from the foregoing, that the two species have undergone very little change during the course of evolution as these species have diverged in relatively recent past and the agglomeration of genetic differences has taken place more or less at the same rate . It is further admitted that much more evidence is needed, especially at the level of DNA based studies, before any generalized correlation between rate of speciation and evolutionary changes.

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Table-1
Random decamer primer sequences used in the present study and their amplification profiles

Primer number	Nucleotide sequence (5'-3')	Amplification profile	References
1	TGATCCCTGG	Initial denaturation at 94°C for 5 min (1 cycle), 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min (1 cycle).	Infante Malachias et al (1999)
2	AGGGCGTAAG		-do-
3	CAGCCCAGAG		-do-
4	GTCCCGACGA		-do-
5	GGTGACGCAG		-do-
6	TGGGGGACTC		-do-
7	GTAGACCCGT		-do-
8	TGCGTGCTTG		-do-
9	CTCTGGAGAC		-do-
10	TCTCCGCTTG		-do-
11	TCGTTCCGCA		-do-
12	GGTGCTCCGT		-do-
13	ACGGATCCTG	Initial denaturation at 94°C for 2 min(1 cycle), 45 cycles of denaturation at 94°C for 1 min,annealing at 35°C for 1min, extension at 72°Cfor 2 min followed by a final extension at 72°C for 6 min (1 cycle).	Xu et al (1998)
14	CCTGATCACC		-do-
15	GGTGATCAGG		-do-
16	CCGAATTCCC		-do-
17	CTCAGTGTCC		-do-
18	CTGGACGTCA		-do-
19	TCGCATCCCT		-do-
20	ACGGTACCAG		-do-

Table-2
The primers used number of fragments amplified, number of monomorphic bands, average heterozygosities and range of size of fragments among *H. pulchra, L. cuprina* and *C. megacephala.*

Primer no.	Sequence 5'-3'	Number of amplified fragments in		Range of amplified fragments in base pair (bp)
		M. domestica n=30	*A. orientalis* n=30	
1	TGATCCCTGG	6(1) [0.229]	5(1) [0.112]	326-2535
2	AGGGCGTAAG	6(2) [0.119]	4(2) [0.087]	236-2163
3	CAGCCCAGAG	6(1) [0.196]	4(1) [0.098]	273-1536
4	GTCCCGACGA	6 [0.284]	3 [0.110]	222-2541
5	GGTGACGCAG	4 [0.276]	2 [0.122]	202-1489
6	TGGGGGACTC	5 [0.231]	4(1) [0.119]	223-1824
7	GTAGACCCGT	0 [0]	0 [0]	0
8	TGCGTGCTTG	6 [0.215]	6(2) [0.116]	121-1998
9	CTCTGGAGAC	8(1) [0.184]	6(1) [0.117]	179-2246
10	TCTCCGCTTG	4 [0.196]	7(2)[0.123]	207-1638
11	TCGTTCCGCA	4 [0.144]	3(2)[0.120]	210-2568
12	GGTGCTCCGT	5(2) [0.135]	5(1)[0.099]	194-2002
13	ACGGATCCTG	6 [0.179]	6(2)[0.126]	186-1336
14	CCTGATCACC	7(1) [0.175]	5(1)[0.110]	246-2067
15	GGTGATCAGG	8(1) [0.197]	5(1)[0.113]	216-2018
16	CCGAATTCCC	7(1) [0.214]	8(3)[0.136]	313-1021
17	CTCAGTGTCC	5 [0.220]	4[0.115]	181-2189
18	CTGGACGTCA	4 [0.219]	6(2)[0.183]	431-1486
19	TCGCATCCCT	8(2) [0.267]	5(1)[0.206]	202-1957
20	ACGGTACCAG	8(2) [0.251]	7(2)[0.183]	244-2088
Mean heterozygosity		**0.197**	**0.139**	

Values under parentheses represent monomorphic bands. Values under square bracket represent heterozygosity. n= sample size.

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