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POLYMORPHISM AMONG DIFFERENT FAMILIES OF DIPTERAN ORDER: COMPARISON OF RAPD DATA

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Abstract: Random amplified polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) data was compared to assess heterozygosity and genetic variability among different members belonging to family Muscidae, Sarcophagidae and Tephritidae. The present review compiles data obtained by ten different RAPD primers. The result shows that *M. domestica* belonging to the family Muscidae have higher heterozygosity value than members belonging to the family Sarcophagidae and Tephritidae which could be depicted by the fact that *Musca* flies have more prominent population thickness.

Keywords: Molecular marker, Muscidae, Primer, RAPD-PCR, Sarcophagidae, Tephritidae.

INTRODUCTION

Dipteran order comprises of insects of great medical, veterinary, forensic and economic importance as the members belonging to this order play a vital role in disease transmission, causing animal tissue myiasis, causing a huge loss of fruits and vegetables as larvae infest wide range of plant species and also members of this order are used to give information related to time along with place of death i.e. in forensic studies (Greenberg, 1971 and 1973; Cornaby, 1974; Jiron and Marin, 1982; Singh *et al.,* 2011; Rawat, 2020).

Now a days, protein based (allozyme) and several DNA based molecular markers (Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD PCR), Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR RFLP), sequencing of different mitochondrial and nuclear genes, Single Strand Conformation Polymorphism (SSCP), Single Nucleotide Polymorphism (SNP), microsatellites) are frequently used for genetic characterization. These have been extensively used to detect intra and inter specific genetic variation, pattern of migration, phylogenetic relationships and population structure among members belonging to different Dipteran families (Zheng *et al.,* 2010; Bajpai and Tewari, 2010a; Singh *et al.,* 2012; Bajpai, 2016a & 2016b; Julsirikul *et al.,* 2017; Park *et al.,* 2018; Bajpai, 2019).

Among all other DNA based methods RAPD-PCR and sequencing of mitochondrial COI gene are more frequently used. RAPD-PCR allows DNA polymorphism by using arbitrary primers, however, sequencing requires prior knowledge of the flanking regions of the gene of interest. RAPD-PCR molecular markers are dominant expression marker in which banding patterns are obtained by using arbitrary primers which amplify numerous regions of the genome (Ali *et al.,* 2004; Jain *et al.,* 2010).

In the present review, an attempt has been made to summarize and analyze the polymorphism by

Table 1: Sequence of primers used, number of amplified fragments, average heterozygosity and range of size of fragments among different families of Dipteran order.

S. no.	Primer sequence 5'-3'	Number of amplified fragments in the Family Muscidae				Number of amplified fragments in the Family Sarcophagidae					Number of amplified fragments in the Family Tephritidae		Range of size of amplified fragments (bp)
		M. domestica (Arail)	*M. domestica* (Prayagraj city)	*M. domestica* (Jhunsi)	*A. orientalis*	*S. ruficornis*	*S. argyrostoma*	*S. dux*	*S. albiceps*	*S. knabi*	*B. cucurbitaceae*	*B. dorsalis*	
1.	TGATCCCTGG	2 (0.25)	2 (0.36)	5 (0.37)	5 (0.11)	2 (0.12)	3 (0.11)	5 (0.38)	2 (0.12)	4 (0.21)	1 (0)	1 (0)	240-2535
2.	AGGGCGTAAG	3 (0.32)	3 (0.38)	3 (0.44)	4 (0.08)	3 (0.09)	4 (0.17)	6 (0.21)	2 (0.08)	2 (0.08)	-	-	237-2163
3.	CAGCCCAGAG	5 (0.37)	2 (0.33)	4 (0.38)	4 (0.09)	5 (0.15)	5 (0.19)	2 (0.06)	3 (0.11)	2 (0.04)	-	-	192-2478
4.	GTCCCGACGA	6 (0.24)	4 (0.27)	6 (0.41)	3 (0.11)	2 (0)	2 (0)	2 (0)	2 (0.28)	3 (0.09)	-	-	214-2541
5.	GGTGACGCAG	5 (0.35)	2 (0.4)	3 (0.37)	2 (0.12)	5 (0.23)	4 (0.14)	6 (0.22)	2 (0.09)	2 (0.09)	-	-	202-1489
6.	TGGGGGACTC	3 (0.27)	4 (0.33)	5 (0.43)	4 (0.11)	5 (0.12)	7 (0.25)	6 (0.24)	3 (0.07)	1 (0)	-	-	196-2450
7.	GTAGACCCGT	5 (0.34)	5 (0.32)	2 (-0.39)	0 (0)	0	0	0	0	1 (0.46)	-	-	144-590
8.	TGCGTGCTTG	3 (0.39)	2 (0.34)	2 (0.43)	6 (0.11)	6 (0.18)	5 (0.13)	3 (0.1)	4 (0.14)	2 (0.07)	4 (0)	4 (0)	100-2039
9.	CTCTGGAGAC	5 (0.21)	4 (0.4)	4 (0.43)	6 -0.11)	2 (0.12)	2 (0.12)	5 (0.12)	1 (0.19)	4 (0.24)	-	-	179-2334
10.	TCTCCGCTTG	5 (0.31)	4 (0.42)	4 (0.42)	7 (0.12)	5 (0.19)	3 (0.13)	3 (0.14)	4 (0.19)	2 (0)	-	-	207-1638
		Malviya *et al*., 2015				Bajpai and Tewari 2010b, Bajpai *et al*., 2011; Bajpai, 2016c; Bajpai, 2016 d					Singh *et al*., 2011		

*values under square bracket represent average heterozygosity.

data obtained from ten different RAPD-PCR primers in members belonging to Muscidae, Sarcophagidae and Tephritidae families of Dipteran order. In Muscidae family three different populations of *Musca domestica* and one species of *Atherigonia orientalis*, in the family Sarcophagidae five different species of *Sarcophaga* namely *S. ruficornis, S. argyrostoma, S. dux, S. albiceps* and *S. knabi* and in the family Tephritidae two different species namely *Bactrocera dorsalis* and *B. cucurbita* were compared.

Comparison of RAPD-PCR data

In the family Muscidae the minimum number of amplified fragments were two and maximum number of amplified fragment were six while in Sarcophagidae family minimum number of scorable bands were two and maximum number of scorable bands were seven and in the family Tephritidae minimum and maximum number of a scorable bands were one and four, respectively. The minimum length of amplified fragment was of 100 base pairs from primer number eight and maximum length of amplified fragment was 2541 base pairs obtained by primary number four.

In the family Muscidae three different populations (flies of Jhunsi, Arail and Prayagraj city region) of *Musca domestica* has been analyzed and the value of heterozygosity ranges from 0.21 to 0.44, however, the heterozygosity value of *A. orientalis* ranges from 0.08 to 0.12, while in Sarcophagidae family heterozygosity value ranges from 0.0 to 0.38. However, in Tephritidae family only two primers are capable of producing banding pattern in both the species; with these two primers only single fragment was scorable by primer one and only four bands were scorable by primer number eight. Both primers

produce monomorphic banding pattern in both the genera therefore, heterozygosity value was found to be zero. Table 1 represents sequence of primers used, number of amplified fragments, average heterozygosity and range of size of fragments amplified among different families of Dipteran order.

A higher estimation of heterozygosity value in *M. domestica* population can be depicted by the fact that this fly is having more prominent population thickness when contrasted with *A. orientalis* or by individuals from other two families, since, those species which are distributed over an enormous zone are liable to increased variety of environmental conditions and in this way they are hereditarily more heterogeneous when contrasted with those species which are available in confined zone. More prominent heterogeneity in housefly additionally makes them ready to endure and effectively adapt up to the distinctive ecological and environmental pressure (Li and Graur, 1999; Santos *et al.,* 2005; Sharma *et al.,* 2009; Malviya *et al.,* 2015).

CONCLUSION

The present review strongly confirms the relevance of RAPD-PCR marker as an important molecular method for unravelling genetic relationship among different members belonging to the family Muscidae, Sarcophagidae and Tephritidae of Dipteran order. Since, from all ten primers the families Muscidae and Sarcophagidae produce scorable banding pattern, however, in Tephritidae family only two primers are capable of producing banding pattern. This could be ascribed by the fact that Muscidae and Sarcophagidae are more closely related as compared to the members of the family Tephritidae.

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