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# RAPD-PCR BASED GENETIC RELATIONSHIP OF MUSCID FLIES (DIPTERA: MUSCIDAE)

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## ABSTRACT

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique has been used in the present study to investigate genetic relationship between the two muscids *viz.,* housefly *Musca domestica* Linnaeus and *Lispe orientalis* (Wiedemann). Tools for Population Genetic Analysis (TFPGA) software, was used to calculate genetic identity and distance, using data matrix prepared by the presence and absence of the bands generated by each primer. Average heterozygosity ranges from 0.156 to 0.301. The Nei's genetic identity was 0.322, which revealed rather low genetic identity between the two species.

**KEY WORDS :RAPD-PCR, *Musca domestica*, *Lispe orientails*, genetic relationship, Muscidae, heterozygosity.**

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## INTRODUCTION

The house fly, *Musca domestica* Linnaeus, is a synanthropic filth fly of cosmopolitan distribution, with great economic and veterinary importance [1, 2]. The flies belonging to genus *Lispe* usually inhabit moist habitats, and are predators feeding on small aquatic insects, and the adults may be instrumental in reducing mosquito populations [3]. The advent of the Polymerase Chain Reaction marked the beginning of a revolution in the molecular biology and synthesis of molecular and evolutionary systematics [4]. Random Amplified Polymorphic DNA, a polymerase chain reaction (PCR) technique, is of immense value for work on anonymous genomes, where only limited quantities of DNA are available. Its cost effectiveness enables one to resolve inter- and intra-specific relationships among insects as these organisms have a relatively large genome size, which increases the probability of finding polymorphism [4-7]. Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) has been extensively used to analyze genetic relationship in several dipterans [8-22]. In the present study, genetic relationship between two muscid species *viz., Musca domestica* Linnaeus and *Lispe orientalis* Wiedemann has been analyzed using RAPD-PCR.

## MATERIALS AND METHODS

Flies were collected using sweep net. DNA extraction, amplification, visualization of gels and interpretation of bands, were carried out following the method of Bajpai and Tewari [9]. A negative control amplification reaction was also performed for each primer. Individuals exhibiting a particular band were assumed to be homozygous or heterozygous for a dominant allele at that locus while the individuals which failed to exhibit a band at that locus were interpreted as homozygous recessive [8]. Average heterozygosity, Nei's genetic identity and distance [23] were calculated by using Tools For Population Genetic Analysis (TFPGA) software [24]. Duplicate PCR reactions for each individual template DNA were performed to test the reproducibility of bands. Genetic relationship between the two species was analyzed by preparing a data matrix for each primer, presence/absence of bands for each primer in 30 individuals from each species.

## RESULTS AND DISCUSSION

The primers used, total number of fragments amplified, number of exclusive fragments amplified by each primer in both species (n), their assignment according to number given in original data (f) and size in base pairs (bp), frequency (%), range of size of amplicons and genetic identity values by each primer in both the species are presented in Table 1.

**Table 1**

***The primers used, total number of fragments amplified, Number of exclusive fragments in both species(n), amplified by each primer, their characterization according to number given in original data (f), their size in base pairs (bp) and their frequency (%), range of size of amplicons and genetic identity values by each primer in M. domestica and L. orientalis.***

| S.No. | Sequence (5'-3') | No. of fragments amplified | No. of exclusive fragments (n) | *M. domestica* | | | *L. orientalis* | | | Length of amplified fragments in base pair | Genetic Identity 1 vs. 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | f | bp | % | f | bp | % | | |
| 1. | TGATCCCTGG | 9 | 6 | 2<br>3<br>4 | 2018<br>532<br>218 | 77.3<br>86.3<br>72.2 | 3<br>5<br>8 | 1512<br>824<br>433 | 81.8<br>77.3<br>86.6 | 218-2589 | 0.203 |
| 2. | AGGGCGTAAG | 5 | 3 | 2 | 667 | 81.8 | 1<br>3 | 979<br>489 | 86.3<br>81.8 | 233-979 | 0.299 |
| 3. | CAGCCCAGAG | 6 | 4 | 3<br>5 | 1102<br>493 | 86.3<br>81.8 | 2<br>4 | 1604<br>967 | 86.3<br>99.0 | 292-2078 | 0.288 |
| 4. | GTCCCGACGA | 8 | 7 | 2<br>3<br>5<br>8 | 2515<br>1532<br>218<br>374 | 77.3<br>81.8<br>86.6<br>77.3 | 1<br>5<br>7 | 2658<br>824<br>622 | 86.8<br>72.3<br>81.8 | 374-2658 | 0.113 |
| 5. | GGTGACGCAG | 7 | 4 | 5 | 688 | 99.0 | 2<br>4<br>7 | 1223<br>965<br>179 | 81.8<br>81.8<br>86.3 | 179-1425 | 0.366 |
| 6. | TGGGGGACTC | 6 | 3 | 3<br>4 | 545<br>418 | 81.8<br>86.8 | 2<br>5 | 616<br>321 | 86.3<br>86.3 | 206-796 | 0.395 |
| 7. | GTAGACCCGT | 8 | 4 | 4 | 503 | 81.8 | 2<br>6<br>8 | 881<br>348<br>121 | 81.8<br>81.8<br>77.3 | 121-1042 | 0.388 |
| 8. | TGCGTGCTTG | 9 | 2 | 1 | 1638 | 86.8 | 8 | 334 | 81.8 | 218-1638 | 0.379 |
| 9. | CTCTGGAGAC | 7 | 4 | 6<br>7 | 498<br>256 | 86.3<br>81.8 | 1<br>2 | 1934<br>1321 | 86.3<br>86.3 | 256-1934 | 0.546 |
| 10. | TCTCCGCTTG | 7 | 3 | 7<br>5 | 318<br>577 | 81.8<br>86.6 | 4 | 889 | 99.0 | 318-1171 | 0.300 |

A total of 71 bands ranging from 121 bp to 2658 bp were observed between the two species. Forty bands were exclusive, out of which 18 and 22 exclusive fragments were observed in *M. domestica* and *L. orientalis*, respectively. Only those fragments were considered exclusive which were species specific and observed in more than 70% individuals. Amplification patterns in both the species, as revealed by primer 1 and primer 4 are depicted in figure 1

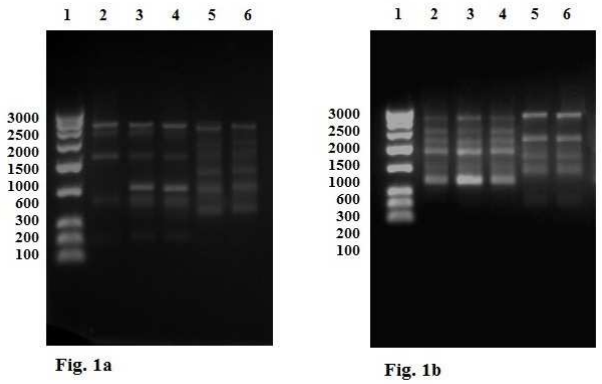


**Figure 1**

***RAPD-PCR banding patterns derived from primer 1 (1a) and primer 4 (1b). Lane 1: molecular weight marker (Low range DNA ruler), Lane 2-4: Musca domestica, Lane 5-6: Lispe orientalis.***

*L. orientalis* showed a higher percentage of monomorphic bands *viz,* 41.3% while in *M. domestica* only 23.0% bands were monomorphic. The mean heterozygosity in the two species ranges from 0.156 to 0.301. Higher mean heterozygosity is observed in *M. domestica*. The genetic identity value ranges from 0.113 to 0.546. Mean genetic identity between *M. domestica* and *L. orientalis* is 0.322. The mean average heterozygosity revealed by the RAPD marker in the two muscid species i.e., *M. domestica* (0.287) and *L. orientalis* (0.142) is slightly greater as compared to other muscid species viz., *M. domestica* (0.163) [25], *Stomoxys calcitrans* (0.070) [26], *and Haematobia irritans* (0.131) [27]. This may be attributed to the fact that RAPD markers are more variable than the other markers [28].The average heterozygosity values obtained in other calyptrate families *viz.,* Calliphoridae, Oestridae and Sarcophagidae ranges from 0.08 to 0.168 which shows that Genetic divergence in these families is rather low [8, 9, 18], as compared to *M. domestica.* However the average heterozygosity in *L. orientalis* was rather low. Thus, it seems that in housefly the heterogeneity is greater than the other muscid flies, and it may be attributed to the greater population density in the environment. Several factors i.e., Environmental changes, genetic drift and population bottleneck play an important role in genetic variation within and among the populations [29]. The genetic variation depends on colonization, host and reproductive pressures such that any species distributed over a great variety of environmental conditions would be genetically more heterozygous as compared to the species of restricted distribution [30, 31, 32]. Presence of variability in housefly is essential for their ability to survive and successfully respond to environmental stresses [20]. The intra-specific genetic identity values in both the species are higher than 0.700, which may be due to the fact that the individuals of the same species represent a narrow genetic pool [33]. Genetic identity values between the two muscid species were low consistent with the fact that *M. domestica* and *L. orientalis* belong to different subfamilies of Muscidae i.e., Musciinae and Coenosiinae, respectively [34].

## CONCLUSION

The RAPD-PCR generates diagnostic markers *viz.*, constant or polymorphic fragments diagnostic for a genus as well as between species within the genus. The data may also be used for elucidating systematic and genetic relationships. In the present study

RAPD-PCR analysis of the two muscid species *viz., M. domestica* and *L. orientalis* reveals that the two species are distantly related as revealed by low genetic identity values.

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