RAPD-PCR-Based Genetic Relationships Among Three Forensically Important Calliphorid Species (Diptera: Calliphoridae)

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Abstract. Molecular genetic markers have been effectively used to analyze genetic relationships and diversity among different groups of dipterans. The emergence of Polymerase Chain Reaction (PCR) facilitated analysis of molecular markers e.g., Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR), has contributed a great deal in resolving the genetic relatedness in many dipterans of medical, veterinary, and economic importance. In the present study, an attempt has been made to explore the phylogenetic relationships among three calliphorid species, namely *Hemipyrellia pulchra* (Weidemann) and *Lucilia cuprina* (Weidemann), *Chrysomya megacephala* (Fabricius), employing Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) technique using twenty random decamer primers. Complete genomic DNA was isolated from the three species and amplified by PCR using twenty random decamer primers. A total of 285 bands ranging from 141 bp to 2648 bp were generated. Tools for population genetic analysis (TFPGA) software was used to calculate genetic identity among the three species. A close relationship among the three species is reflected by high values of mean Genetic identity (0.661–0.713).

Keywords: RAPD-PCR · Calliphoridae · Genetic relationship · Molecular markers · TFPGA

1 Introduction

Calliphorids are a genetically diversified group of dipterans having medical, forensic and veterinary relevance as they may act as vectors of some pathogens and some species of them are also reported to cause myiasis in vertebrates. *Chrysomya megacephala*, commonly known as the oriental latrine fly or oriental blue fly, and *Lucilia cuprina*, commonly known as the Australian sheep blowfly are of great economic and veterinary importance [1, 2]. The emergence of the Polymerase Chain Reaction conducted a revolution in molecular biology and introduced the era of molecular and evolutionary systematics [3]. Random Amplified Polymorphic DNA, a polymerase chain reaction (PCR) technique, is an exemplary tool for working on anonymous genomes, with very scanty quantities of DNA. Since insects have a large genome size with high levels of polymorphism, RAPD-PCR proves to be a cost-effective technique to unravel inter and

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intra-specific relationships among them [3–6]. RAPD-PCR has been substantially used to explore genetic relatedness in dipterans [7–21]. In the present study, genetic relatedness among three calliphorid species, namely *Hemipyrellia pulchra* (Weidemann), *Lucilia cuprina* (Weidemann), and *Chrysomya megacephala* (Fabricius), by RAPD-PCR using twenty arbitrary decamer primers.

2 Materials and Methods

The present investigation was carried out on three laboratory-reared/maintained Calliphorid species *viz*., *Hemipyrellia pulchra* (Weidemann), *Lucilia cuprina* (Weidemann), and *Chrysomya megacephala* (Fabricius). For Genomic DNA isolation individuals were homogenized in extraction buffer (50 mM EDTA, 0.1 M Tris, 0.2 M sucrose, and 0.5% SDS (pH-8.0)) and digested with RNase with an incubation of 30 min at 37 °C. Then DNA was phenol/chloroform (1:1) extracted using Isoamyl alcohol, precipitated with ethanol, and suspended in 100 μl Tris: EDTA buffer (pH-8.0) Quantities of the extracted DNA were estimated using a UV-Vis spectrophotometer (Elico). DNA was quantified by examining the ratio of the optical densities at 260 nm and 280 nm. The DNA concentration (ng/μl) was calculated by using the formula DNA = OD_{260}X2500 [13].

The quality of DNA quality was also estimated by electrophoresis of the extracted DNA sample on 1% agarose gel. Twenty decamer primers were used to amplify the genomic DNA of the three species (Table 1) [14, 15]. A molecular weight marker (Low range DNA ruler; 100 bp–3000 bp) was loaded in the first well and the samples were loaded in the rest of the wells of the gel in a horizontal electrophoresis chamber. Electrophoresis was performed at 50 V for 2 h 30 min. The gels were visualized and photographed under UV illumination under a BIOVIS Gel Documentation System, to assess the quality of extracted DNA and analyzed with Biovis V4 1D Gel. A data matrix was created using the presence/absence of each band from 30 individuals of the three species which was employed to analyze average heterozygosity with Tools for Population Genetic Analysis (TFPGA) Software [16]. A confirmation reaction was also performed for each primer through negative control amplification. Individuals were considered as homozygous or heterozygous for a dominant allele at that locus exhibiting a particular band while the individuals were assumed as homozygous recessive which failed to exhibit a band at that locus [7]. Average heterozygosity, Tools For Population Genetic Analysis (TFPGA) software was employed to estimate Nei's genetic identity and distance [22, 23]. The reproducibility of bands was checked through duplicate PCR reactions.

3 Results and Discussion

The primers used, the total number of fragments amplified, number of exclusive fragments amplified by each primer in the three species (n), range of size of amplified fragments have been depicted in Table 1. Table 2 represents Genetic identity values by each primer in the three species.

RAPD-PCR is a methodical technique to analyze genetic polymorphisms. The technique is based on the use of random decamer primers to detect the polymorphisms within

the genomic DNA. As RAPDs are dominant expression markers, the presence or absence of amplicons provides the idea of polymorphisms for a single locus [7]. RAPD-PCR has been successfully used in systematics and population genetic studies of dipterans of medical, veterinary, and economic importance [8, 12, 13, 18, 21].

In the present study, twenty random decamer primers amplified a total of 285 reproducible fragments ranging from 143 bp to 2642 bp among the three species. Eighty-nine fragments were amplified in *H. pulchra* out of which twenty (22.5%) fragments were monomorphic. In *L. cuprina* ninety-five fragments were amplified out of which twenty two (24.72%) fragments were monomorphic. In *C. megacephala* eighty fragments were amplified out of which eighteen (21.05%) fragments were monomorphic. Species specificity and presence in more than 70% of individuals was the criterion to consider a fragment as exclusive. Amplification patterns in the three species, as revealed by primer 4 and primer 20 are depicted in Figs. 1 and 2 respectively.

A higher percentage of monomorphic bands viz, 24.72% was shown in *L. cuprina* while in *H. pulchra* and *C. megacephala* 22.5% and 20.05% bands were found to be monomorphic respectively. The range of mean heterozygosity was found to be 0.127–0.160 in the three species. Higher mean heterozygosity is observed in *C. megacephala.* The genetic identity value ranges from 0.526 to 0.753. Mean genetic identity among the three species ranges from 0.661–0.713. The mean average heterozygosity in the three calliphorid species is greater as compared to other calliphorid species due to more variability of RAPD markers [24]. Thus, it seems that higher values of heterogeneity are reflected by greater population density as well as environmental changes, genetic drift, and population bottleneck within and among the populations [25–28]. The intra-specific genetic identity values in the three species (greater than 0.526), attribute that a narrow genetic pool is represented by the individuals of the same species 33. Genetic identity values between *H. pulchra* and *L. cuprina* were found to be higher as both the species belong to the same subfamily of Calliphoridae i.e., Lucillinae.

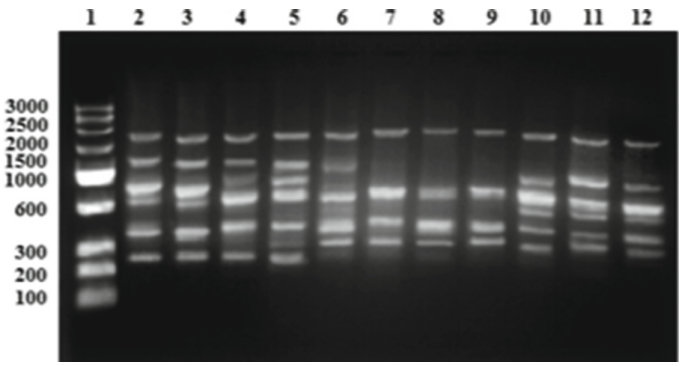

Fig. 1. RAPD patterns in the three calliphorid species as revealed by primer no. 4. Lane 1: Molecular weight marker (100–3000 bp), Lane 2–5: *H. pulchra*, Lane 6–9: *C. megacephala*, Lane 10–12: *L. cuprina*

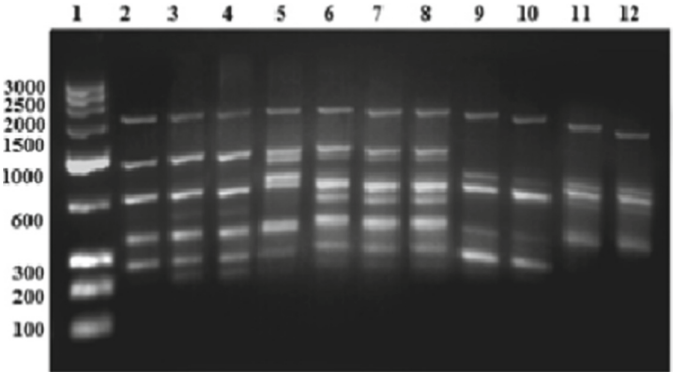

Fig. 2. RAPD patterns in the three calliphorid species as revealed by primer no. 20. Lane 1: Molecular weight marker (100–3000 bp), Lane 2–5: *H. pulchra*, Lane 6–9: *C. megacephala*, Lane 10–12: *L. cuprina*

Table 1. Represents Sequences of the twenty decamer primers, No. of amplified fragments, No. of mono morphic bands (in parentheses), Average heterozygosities [in bold] in the three calliphorid species, and the range of amplified fragments in base pair

Primer no.	Sequence 5'-3'	Number of amplified fragments in			Range of amplified fragments in base pair (bp)
		H. pulchra n = 30	*L. cuprina* n = 30	*C. megacephala* n = 30	
1	TGATCCCTGG	6(1) **[0.121]**	5(1) **[0.117]**	1	TGATCCCTGG
2	AGGGCGTAAG	3(2) **[0.099]**	3 (2) **[0.088]**	2	AGGGCGTAAG
3	CAGCCCAGAG	5(2) **[0.116]**	4(1) **[0.103]**	3	CAGCCCAGAG
4	GTCCCGACGA	6(2) **[0.134]**	5(1) **[0.112]**	4	GTCCCGACGA
5	GGTGACGCAG	4(1) **[0.167]**	3(1) **[0.140]**	5	GGTGACGCAG
6	TGGGGGACTC	5(2) **[0.165]**	5(1) **[0.144]**	6	TGGGGGACTC
7	GTAGACCCGT	0 **[0]**	0 **[0]**	7	GTAGACCCGT
8	TGCGTGCTTG	5(2) **[0.156]**	8(2) **[0.118]**	8	TGCGTGCTTG
9	CTCTGGAGAC	5(1) **[0.122]**	6(1) **[0.122]**	9	CTCTGGAGAC
10	TCTCCGCTTG	3(1) **[0.137]**	8(2) **[0.132]**	10	TCTCCGCTTG

(*continued*)

Table 1. (*continued*)

Primer no.	Sequence 5'-3'	Number of amplified fragments in			Range of amplified fragments in base pair (bp)
		H. pulchra n = 30	*L. cuprina* n = 30	*C. megacephala* n = 30	
11	TCGTTCCGCA	6(1) **[0.119]**	6(2) **[0.115]**	11	TCGTTCCGCA
12	GGTGCTCCGT	3 **[0.121]**	3 **[0.113]**	12	GGTGCTCCGT
13	ACGGATCCTG	3 **[0.138]**	3 **[0.131]**	13	ACGGATCCTG
14	CCTGATCACC	6(2) **[0.129]**	4(1) **[0.121]**	14	CCTGATCACC
15	GGTGATCAGG	6(1) **[0.134]**	5(1) **[0.123]**	15	GGTGATCAGG
16	CCGAATTCCC	6 **[0.156]**	5(1) **[0.133]**	16	CCGAATTCCC
17	CTCAGTGTCC	5(1) **[0.169]**	4 **[0.139]**	17	CTCAGTGTCC
18	CTGGACGTCA	6(2) **[0.196]**	7(2) **[0.161]**	18	CTGGACGTCA
19	TCGCATCCCT	2 **[0.252]**	4 **[0.215]**	19	TCGCATCCCT
20	ACGGTACCAG	5(1) **[0.228]**	7(1) **[0.207]**	20	ACGGTACCAG
	Mean heterozygosity	**0.138**	**0.127**		Mean heterozygosity

4 Conclusion

The RAPD-PCR produces predictive markers in the form of constant or polymorphic fragments characteristic of a genus as well as species. The data thus produced may also be used to interpret systematic and genetic relatedness. In the present study RAPD-PCR analysis of the three calliphorid species viz., *Hemipyrellia pulchra* (Weidemann) and *Lucilia cuprina* (Weidemann), *Chrysomya megacephala* (Fabricius), reveals that *Hemipyrellia pulchra* (Weidemann) and *Lucilia cuprina* (Weidemann), are closely related to each other as compared to *C. megacephala* as revealed by low genetic identity values.

Table 2. Represents Nei's Genetic Identity values by each primer as calculated by TFPGA software among the three calliphorid species

Primer no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
H vs. L	0.701	0.725	0.741	0.695	0.689	0.731	0.699	0.709	0.715	0.711	0.746	0.687	0.677	0.739	0.710	0.685	0.751	0.721	0.682	0.753	0.713
H Vs c	0.678	0.703	0.596	0.667	0.736	0.726	0.621	0.616	0.673	0.557	0.773	0.526	0.631	0.593	0.605	0.728	0.802	0.659	0.605	0.707	0.661
L vs. C	0.706	0.711	0.703	0.679	0.655	0.685	0.694	0.679	0.685	0.673	0.666	0.699	0.682	0.656	0.727	0.666	0.672	0.636	0.665	0.718	0.683

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