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Utility of Random Amplified Polymorphic DNA (RAPD) In Forensic Entomology

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ABSTRACT

Forensic entomology is the study of insects found around the crime scene and are helpful for the estimation of time elapsed since death of a person i.e., post mortem interval (PMI). There are number techniques available for the identification of insect species and Random Amplified Polymorphic DNA (RAPD) is one of those. RAPD is a useful technique designed for the detection of sequence polymorphism in organisms where previous nucleotide sequence data is not available. RAPDs are based on random priming using short primers of arbitrary sequence that act as both forward and reverse primers in the PCR reaction.

Keywords: PMI, RAPD, DNA, PCR

I. INTRODUCTION

Forensic entomology is a science, which applies knowledge of insects (and other arthropods) to civil proceedings and criminal trials (Turchetto and Vanin, 2004). Although it has come of age as a science only in the last 40 years, it is a field with a long history. The first documented case of forensic entomology took place in 13th century China. In the book, "Hsi Yuan Chi Lu" (one possible translation is "the washing away of wrong") (McKnight, 1981), Chinese criminalist Sung Tz'u reported a case in which insects were used to identify a murderer. A murder was committed by slashing, and all villagers were ordered to bring their sickles to a single location. The sickles were laid on the ground, and flies were attracted to a single sickle, presumably responding to traces of tissue and blood. On the basis, the owner of the sickle broke down and confessed.

Random Amplified Polymorphic DNA (RAPD) is a method which uses non-specific primers and the PCR products come from many areas of the specimen sample of DNA. The advantages of using RAPD analysis are: the process is quick; the room for experimental error and sample contamination during the analysis is reduced; the chemicals for RAPD analysis have a long shelf-life; and a large amount of information is recovered. There are some disadvantages of using RAPD analysis of insect DNA, however, since the signatures gained for different species will not be standardized; no national or international databases exist from which to compare RAPD profiles of the insect species; and no statistical data are available in order to exclude chance when interpreting the results (Benecke, 1998b; Gennard, 2007). The primers produce a series of bands that may be visualized using agarose gel electrophoresis and each of the products represents a single genetic locus (Hill and Crampton, 1994).

II. RAPD IN FORENSIC ENTOMOLOGY

Stevens and Wall (1995) evaluated the use of the random amplified polymorphic DNA (RAPD) polymerase chain reaction to characterize individual *Lucilia sericata* Meigen from southern England . They investigate some simple techniques which allowed the preservation and extraction of DNA to be optimized without the complications of transporting liquid nitrogen. The RAPD results show that closely related *L. sericata*, including those from a single strike, can be readily distinguished from each other on the basis of their RAPD profiles resolved using electrophoretic analysis; profiles were defined with ten random primers. Analysis of these RAPD data using a similarity coefficient method and a recently developed randomization test to detect the non-random association of alleles at different loci, allowed the genetic homogeneity of *L. sericata* within southern Britain to be explored. This study shows that while a number of factors can complicate the use and interpretation of RAPD fragments as genetic markers, RAPD fingerprinting can be a valuable technique for studies of intraspecific genetic variation in *L. sericata*.

Stevens and Wall (1997) studied intraspecific genetic variation in two species of calliphorid blowfly, *Lucilia sericata* and *Lucilia cuprina*, by random amplified polymorphic DNA (RAPD) analysis and mitochondrial DNA (mtDNA) sequencing. They showed that these species are economically important facultative ectoparasites of sheep. They have used numerical analysis of RAPD fragment data to investigate genetic variation in *L. sericata* across Europe and in both *L. sericata* and *L. cuprina* worldwide. No evidence of genetic isolation within *L. sericata* was observed, despite the geographic separation of the populations studied. Their finding was supported by a lack of variation in mtDNA sequences from a corresponding global sample of *L. sericata*. For *L. cuprina* distinct patterns of genetic variation, possibly related to geographical isolation, were detected in the RAPD data and the mtDNA sequences.

The first application of RAPD to differentiate forensically relevant insects was done by Benecke (1998). He found the RAPD technique unsuitable for species identification due to many disadvantages like high information density; variable patterns between most (if not all) known species and different peaks, heights and shapes. Few more limitations of RAPDs are, non genetic variation in the analysis of the progeny of controlled mating, indicating some artifacts in priming, the co-migration of products of equal size and homologous loci that are difficult to identify (Palumbi, 1996). So, the applicability and utility of RAPDs in forensic casework is not that much convincing.

RAPD has been successfully applied to study the genetic structure of endangered populations (Zhou *et al.* 2000) interspecific study (Tiple *et al.* 2009) and gene flow between populations (Hoole *et al*., 1999). Earlier RAPD was successfully applied for molecular ~ 546 ~ characterization of two species of butterflies belonging to the family Pieridae (Sharma et al. 2003). By using different primers, polymorphisms at many loci can be detected between species and populations. Therefore, RAPD-PCR analysis can increase the resolution of genetic variations. RAPD has been widely used in the determination of population structure without prior knowledge of DNA sequences on the basis of RAPDs, genetic polymorphisms in natural populations (Haag *et al.* 2003).

Bajpai (2016a) used Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique 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. Bajpai (2016b) again carried out study based on Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) to establish genetic relatiohsip between three forensically important sarcophagids viz., *Sarcophaga ruficornis* (Fab.), *S. argyrostoma* (R. D.) and *S. dux* (Walker).

RAPD is the easiest and inexpensive method for laboratory and have found a wide range of applications mainly due to the speed, cost and efficiency of the

RAPD technique to generate numbers of markers in a short period compared with previous methods. Despite the reproducibility problem, the RAPD method will probably be important for the identification of forensically important species.

III. REFERENCES

[1]. Turchetto, M. and Vanin, S. (2004). Forensic entomology and climatic change. Forensic Science International, 146 (Suppl.): 207-209.

[2]. McKnight, B.E. (1981) The Washing Away of Wrongs : Forensic Medicine in Thirteenth Century China, trans. S. Tzu., University of Michigan, Ann Arbor, pp. 196.

[3]. Benecke, M. (1998) Random amplified polymorphic DNA (RAPD) typing of necrophageous insects (Diptera, Coleoptera) in criminal forensic studies: validation and use in practice. Forensic Science International, 98: 157-168.

[4]. Gennard, D. E. (2007) Forensic entomology: An introduction. John Wiley & Sons Ltd, West Sussex, England, pp. 224.

[5]. Hill, S. and Crampton, J. (1994) DNA-based methods for the identification of insect vectors. Annals of Tropical Medicine and Parasitology, 88: 227-250.

[6]. Stevens JR, Wall R (1995) The use of randomly amplified polymorphic DNA (RAPD) analysis for studies of genetic variation in populations of the blowfly Lucilia sericata (Diptera: Calliphoridae) in southern England. Bulletin of Entomological Research 85:549–555.

[7]. Stevens, J. and R. Wall (1997). Genetic variation in populations of the blowflies, Lucilia cuprina and Lucilia sericata (Diptera: Calliphoridae). Random amplified polymorphic DNA analysis and mitochondrial DNA sequences, Biochematics Systematics and Ecology, 25:81–97.

[8]. Palumbi, S.R. (1996). Nucleic acids II: The polymerase chain reaction. In: Molecular Statematics, Hillis, D.M., Moritz, C. and Mable, B.K. (Eds.), Sinauer Associates, Sunderland, Mass.

[9]. Zhou X, Faktor O, Applebaum SW, Coll M. (2000). Population structure of the pestiferous moth Helicoverpa armigera in the Eastern Mediterranean using RAPD analysis, Heredity, 85: 251-256.

[10]. Tiple AD, Khurad AM, Padwad SV (2009). Genetic relationships among some Lycaenidae butterflies as revealed by RAPD analysis. Cytologia, 74(2):165-169.

[11]. Hoole JC, Joyce DA, Pullin A.S. (1999). Estimates of gene flow between populations of the swallow tail butterfly Papilio machaon in Broadland, UK and implications for conservation. Biological Conservation,89(3):293-299.

[12]. Sharma V.L., Gill T.K., Sharma S., Badran A.L.A., Sobit R.C. (2003). RAPD-PCR in two species of Catopsilia (Pieris, Lepidoptera). Caryologia, 56(2):219-222.

[13]. Haag, L., Karen, A.M., De Araujo, Zaha, A. (1993). Genetic structure of natural population of Dryasiulia (Lepidoptera: Nymphalidae). General Biochemistry, 31:449-460.

[14]. Bajpai, N. (2016a). Random amplified polymorphic DNA based characterization of flesh flies. Journal of Entomology and Zoology Studies, 4(5): 857-859.

[15]. Bajpai, N. (2016b). RAPD-PCR based characterization of sarcophagid flies. The Journal of Zoology Studies, 3(5): 20-23.