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Molecular Characterization of Butterflies and Its Significances in Taxonomy

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Abstract

The present study was aimed to highlight the use and significances of molecular characterization of insect especially butterflies. The order Lepidoptera is largest and important group of class insecta. The insects are larger in terms of numbers of species and many species of these insects are still not satisfactory. The traditional or morphological method of identification of butterflies is usually based on the wing patterns and this method is not considered as effective to fulfil the need of proper and fast identification. Various authors have identified butterfly's species by their morphology, but it's possible that some small invisible change in morphological characteristics may remain unnoticed. The molecular techniques provide an important tool that ease the assessment of genetic diversity and facilitate genotyping, classification, inventorying and phylogenetic studies.

Keywords: Molecular, Characterization, Butterflies, Significances, insecta

Introduction

Lepidopterans are regarded as one of the important component of biodiversity^[17] and are the second largest order among insects made up of approximately 1, 50,000 species so far known as per literature. These include moth (Heterocera) and butterflies (Rhopalocera) of which 70,820 are butterflies according to several reports^[22]. Apart from their aesthetic appeal, they are good pollinators. Butterflies are highly sensitive to environmental changes and are delicate creatures. Butterflies and moths offer good opportunities for studies on population and community ecology^[18]. The biodiversity, taxonomy, geographic distribution and status of many species of butterflies are relatively well known. Further, butterflies are good biological indicators of habitat quality as well as general environmental health^[16, 12, 19] as many species are strictly seasonal and prefer only particular set of habitats^[14].

The butterflies are one of the important and most commonly seen insects of the order Lepidoptera. The order Lepidoptera is an economically important group of insects as their larvae exclusively feed on plants. This group is characterized by the high chromosome number and small size of chromosomes that make differentiation of the species difficult at the cytogenetic level^[20]. In India about 1,501 species of butterflies are present^[11]. Some of the species are easy to identify and categorize, while many other species are still unidentified. Although many studies on morphological, ecological and molecular attributes of several species of butterflies from all over the world are available, very little is known about the Indian species^[25]. Morphological identification of butterflies is usually based on the wing patterns^[4, 31, 15]. These factors make morphological criteria not a preferred way for a very accurate differentiation of these species. Various authors have identified butterfly's species by their morphology, but it's possible that some small invisible change in morphological characteristics may remain unnoticed. The molecular techniques provide an important tool that ease the assessment of genetic diversity and facilitate genotyping, classification, inventorying and phylogenetic studies^[2]. There are many molecular techniques available such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and arbitrary fragment length polymorphism (AFLP) etc. to characterize the butterflies at molecular level. RAPD marker is well suited for use in the large sample throughout systems required for population genetics and studies of biodiversity^[28]. RAPD has been successfully applied to study the genetic structure of endangered populations^[35] interspecific study^[26] and gene flow between populations^[8]. Earlier RAPD was successfully applied for molecular

characterization of two species of butterflies belonging to the family Pieridae^[21]. 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 populations⁷. Molecular technologies provide new ways to study population diversity as well as to differentiate closely related species³⁰. The scientific study was conducted on the genetic structure of endangered populations in the Cranberry Fritillary, *Boloria aquilonaris* (Lepidoptera: Nymphalidae) by using molecular tools^[27].

RAPD markers are very well suited for use in insect phylogeny areas like the detection of genetic variation among populations as well as the identification of closely related species^[1]. RAPD can find wide use in their identification and differentiation of closely related species and populations within species^[32, 35]. Genetic polymorphism revealed is of great importance in species diagnostics as the pattern of bands revealed by RAPD-PCR are often species specific. Molecular techniques have also been used to clarify phylogenetic relationships among parasitic Hymenoptera^[36] and to facilitate ecological studies on parasites and predators used in classical, augmentative and conservation biological control programs.

The study was carried out on the butterfly composition in Mussoorie (Uttarakhand) and prepared a checklist of 66 species of lycaenid butterflies^[23]. A study was made on the habitat selection and community structure of butterflies in a moist deciduous forest of Uttarakhand^[10]. An entomological survey has been made at Gurukula Kangri University Campus, Haridwar to record the species composition of butterflies^[13]. During this study a total of 179 individuals belonging to 25 species and 04 families were reported. The Nymphalidae was the most dominant family in terms of number of species and represented by 10 species followed by Pieridae (09), Danaidae (04) and Papilionidae (02). The Indian sub continent (CISC) has about 1439 species of butterflies out of which 100 species are endemic to it and at least 26 taxa are today globally threatened as per the IUCN (1990). Butterfly communities have also been studied along altitudinal gradients in the Pindari area of the Nanda Devi Biosphere Reserve in the Bageshwar district of Uttaranchal⁹.

RAPD-PCR provides a very versatile and widely applied biotechnology technique in entomology. The RAPD-PCR technique is extremely useful for rapid identification of genetic polymorphisms in Lepidopteran because of the reproducibility of the results for each of the species. RAPD techniques in the field of biology helped us to establish genetic relationship between the members of different taxonomic categories. Applicability of museum specimens of butterflies through RAPD analysis has been demonstrated^[34]. By the use of RAPD analysis inter specific hybridization between *Parnassius nomion* and *Parnassius bremeri* has been studied. Recently developed molecular marker techniques provide an important tool that ease the assessment of genetic diversity and facilitate genotyping, classification, inventorying and phylogenetic studies^[3]. Using RAPD-PCR, genetic polymorphisms and genetic diversity in natural populations between species of Nymphalidae have been studied^[6]. The amplification pattern obtained by RAPD primers were considered to be ideal for identification of species²⁴. Three DNA-based approaches have been utilized for mosquito species identification: hybridization assay based on species-

specific sequence^[5], randomly amplified polymorphic DNA^[29] and tests based on known, variable region. On the other hand, the RAPD assay is less expensive and overcomes some technical limitations of RFLPs. The RAPD markers have become the most common yardsticks for measuring genetic differences between individuals, within and between related species or population.

Conclusion

From above different studies it has been concluded that conservation of ecologically and environmentally significant species of butterflies needs proper identification. The morphological method of identification needs large number of experts and time but the molecular techniques are much efficient in proper identification within short period of time. Molecular techniques include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and arbitrary fragment length polymorphism (AFLP) etc. to characterize the butterflies at molecular level. RAPD marker is well suited for use in the large sample throughout systems required for population genetics. These techniques are also useful in insect phylogeny areas like the detection of genetic variation among populations as well as the identification of closely related species. Molecular techniques have also been used to clarify phylogenetic relationships among parasitic insects and relationship between parasites and predators.

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