Advances in Researches on Genetic Diversity of *Lepidoptera* Insects

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Abstract This paper reviewed advances in researches on genetic diversity of Lepidoptera insects from chromosome polymorphism, protein polymorphism, and DNA polymorphism, and stated that DNA sequence variation will become main points of researches about genetic diversity.

Key words Lepidoptera insects, Genetic diversity, Advances in researches

1 Introduction

Biodiversity is a general name of species and their genetic variation and ecosystems of all life forms, including plants, animals, fungi, microorganisms in a given time and area. It is a material base for the survival and development of human society. It includes four levels: genetic diversity, species diversity, ecosystem diversity and landscape diversity. Genetic diversity is the basis and core of biodiversity. It is widely used in various biological fields. In a broad sense, genetic diversity refers to the sum of the genetic information contained in the biological individuals on the earth, manifested in the genetic variability of the three levels (molecules, cells and individuals). In a narrow sense, it mainly refers to the changes in intracellular genes[1]. Genetic diversity and genetic variation are widely found in various organisms and are the fundamental driving force of biological evolution. It mainly includes chromosome polymorphism, protein polymorphism, and DNA polymorphism. In this study, we reviewed advances in researches on the genetic diversity of *Lepidoptera* insects and believed that these detection technologies can comprehensively reveal the genetic diversity of *Lepidoptera* insects and will have broad application prospect.

2 General situations of advances in researches on genetic diversity of *Lepidoptera* insects

2.1 Current situations of researches of *Lepidoptera* insects

Lepidoptera belongs to the Arthropoda, Hexapoda, Insecta. It is an order of insects that includes moths and butterflies. In one generation, *Lepidoptera* insects experience 4 stages of life cycle: eggs, larvae, pupa, and adult. *Lepidoptera* insects are numerous, the known species exceed 255000, widely distributed around the world. With the extensive application of modern biotechnology, researches on *Lepidoptera* insects are extensive, including phylogenetic evolution, identification of relative species, and genetic variation of population[2-3].

2.2 General situations of advances in researches on genetic diversity of *Lepidoptera* insects

2.2.1 Chromosome polymorphism. The order *Lepidoptera* is the second largest order of insects. However, the researches on biology of insect cell chromosomes and mitosis of cells lag behind and hinder further researches and application of insect cells[4]. Zhang Xin *et al.*[5] analyzed the chromosomes of seven *Lepidoptera* insect cell lines, obtained necessary colchicine concentration and treatment time for analysis of various chromosomes and low permeability concentration; chromosomes of seven *Lepidoptera* insects show typical karyotype characteristics of continuous cells of *Lepidoptera* insects. Generally, the number of chromosomes of *Lepidoptera* insects is 31, and sex chromosome is XO or ZW. But there is exception, for example, silkworm $2n = 56$, Hepialus armoricanus $n = 28$, Rondotia menciana Moore $n = 28$, Rondotia menciana Moore $n = 22$, sex chromosome is XO type. There is still controversy about the sex chromosome. It is generally believed that the male chromosome is 27AA + ZZ, female chromosome is 27AA + ZW, it is ZW type decision. The chromosome of *Gynaephora* is special. Liu Zhenkui *et al.*[6] studied Gynaephora menyuanensis, Gynaephora qumalaiensis and Gynaephora qinghaiensis, and found that the number of chromosome is varied in 9 - 107.

2.2.2 Protein polymorphism. Protein polymorphism includes amino acid sequence analysis and isoenzyme electrophoresis analysis. The latter method is convenient and rapid, and the cost is not high, and has become an important means to analyze protein polymorphism.

Tong Zhenxiang *et al.*[7] investigated the distinctive changes in esterase isoenzymes in the larvae during the different development stages of the larvae; there are certain differences between esterase isoenzyme activity and the number of enzyme bands of larvae in different development stages. In full appetite stage, the number of enzyme bands is large and high and the expression is related to growth and development of larva; Shen Wenbiao *et al.*[8] found there are 10 same enzyme bands in different stages of larva through studying the bands of esterase isoenzymes. Xu Guang *et al.*[9] detected 13 allozymes of Helicoverpa armigera Hubner using polyacrylamide gradient gel electrophoresis, and analyzed the genetic variation of 9 allozymes. The results showed that

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there is high genetic polymorphism in the population of *Helicoverpa armigera* Hubner, but the genetic differentiation between different populations is low. There is no obstacle to gene flow in populations. It can be inferred that migration hinders the genetic differentiation between different geographical populations.

2.2.3 DNA polymorphism. With the rapid development of the molecular biology technology, DNA polymorphism has become the most effective genetic analysis method. DNA polymorphism detection methods mainly include RAPD technology, DNA fingerprinting technology, and DNA sequence analysis.

(i) RAPD technology. RAPD technology is PCR based molecule technology that can be used to analyze polymorphism of the genome of the entire unknown sequence. It takes genomic DNA as a template, and a single synthetic random polymorphic nucleotide sequence (9 – 10 bp) as a primer to carry out PCR amplification under the action of Taq enzyme. It uses electrophoresis to separate various lengths of DNA, and make separation and identification of DNA polymorphism generated from organisms. The polymorphism of the amplified products reflects the genomic polymorphism.

At present, RAPD technology has been widely used in the study of biological species identification, pedigree analysis and evolutionary relationship. Sun Shan *et al.* [10] studied the differentiation of Asian maize borers by RAPD technology and found that different t geographical populations of Asian maize borers in China have generated certain degree of differentiation, which is largely connected with geographical isolation, indicating that Asian maize borers may not have the ability of remote migration. Using AFLP technology, Yuan Yiyang *et al.* [11] studied genetic diversity and genetic structure difference of *Dendrolimus tabulaeformis* Tsai et Liu in different environmental conditions. The results showed that the tree growth status is an essential factor influencing the genetic diversity of *Dendrolimus tabulaeformis* population in *Pinus tabulaeformis* populations, and the gene flow size of *Dendrolimus tabulaeformis* is negatively correlated with the species diversity of *Pinus tabulaeformis*. Taking larvae of Holcocerus hippophaecolus as material, Chen Min *et al.* [12] established a set of optimized Holcocerus hippophaecolus AFLP molecular marker system and obtained clear fingerprinting through comparative study of key factors in AFLP experiment, including enzyme cut and link-up, pre-amplification, and selective amplification.

(ii) DNA fingerprinting analysis. DNA fingerprinting analysis is a means of detecting genetic variation by using shorter repeated sequence in the genome as markers. Using DNA fingerprinting analysis method, Zhang *et al.* [13] explored the genetic relationship between eight species and subspecies of the genus Dendrolimus. In 13 random primers, 168 polymorphic molecular markers were detected in 8 species of Dendrolimus. Their results are consistent with conclusions obtained from DNA fingerprint and sex pheromones. Using ISSR markers, C. Luque *et al.* [14] built genomic fingerprints of 6 species of Noctuidae of *Lepidoptera* insects; Kumar *et al.* [15] evaluated the genetic variation of 28 populations of Indian rice pests. Using 6 primers anchored by 5' and 3' end, Reddy *et al.* [14] amplified individuals of different populations of silkworms and found that polymorphic bands account for 77% of the total bands[16]; using ISSR-PCR method, Dai Lingyan *et al.* [17] analyzed gene flow of bollworms in 6 cotton production areas, screened 15 primers and amplified 138 DNA bands, and the polymorphic bands account for 90.6%. Gao Baojia *et al.* [18], with the aid of ISSR technology, analyzed and measured the genetic variation of 10 geographical populations of Dendrolimus spectabilis, *Dendrolimus tabulaeformis*, and Dendrolimus superans, discussed the relationship between genetic differentiation and geographical conditions, and the clustering results indicated that there is certain correlation between genetic distance and geographical distance of different areas of *Dendrolimus tabulaeformis*.

(iii) DNA sequence analysis. DNA sequence variation is the most ideal way for revealing genetic diversity. With the reduction of sequencing costs, using sequencing methods to study biological genetic diversity has become the mainstream technology. DNA sequence analysis is mainly aiming at the partial or full sequence of partial genes of rDNA and mtDNA, such as cytochrome b, COI, COII and other genes, these genes have genetic conservatism, and have great significance for biological evolution research.

With the rapid development of DNA sequencing technology, DNA sequence analysis is playing an increasingly important role in the study of genetic diversity. Chen *et al.* [19] determined the Cyt b gene sequence of five kinds of Parnassius of *Lepidoptera* and carried out systematic evolutionary analysis. This lays an important foundation for the study of DNA diversity testing technology for rare species. Chen *et al.* [20] performed cloning, sequencing and molecular phylogenetic analysis of the mitochondrial COII gene of eri-silkworm. Chen Na *et al.* [21] determined the partial sequence of the mitochondrial 16S rRNA gene of 27 species of Nymphalidae butterflies and 2 species of Danaidae butterflies, established the phylogenetic tree of Nymphalidae, and discussed the phylogenetic relationship between main populations of the Nymphalidae.

3 Research prospects

Genetic diversity is an essential part of biodiversity, and every species has its own unique gene pool and genetic organization, and the diversity of species reflects the diversity of genes. It is the most direct and simple way to detect the diversity of the *Lepidoptera* insects from the morphology or phenotypic traits. However, because phenotypic traits are subject to environmental factors, it is required to make further and deep researches from chromosome level, isoenzyme or allozyme level, and DNA level, to understand genetic variation of populations in a more accurate and detailed manner. In sum, the detection of genetic diversity should be established at different levels. Various methods for detecting genetic diversity should be based on actual conditions. With the great reduction in gene sequencing costs, DNA gene sequencing research will become the mainstream of genetic diversity research.

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Under the optimal process, the taurine content reached 117.8 mg/g. In the experiment, we first used cheap rice as material, and added the precursors cysteinyl acid and methionine for taurine synthesis. Under multi-strain combined action, the taurine content in peptide feed was greatly improved, which provided a good practical value for the wide application of plant-derived proteins in the feed industry.

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