生物多样性研究丛书

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Principles and Methodologies of Genetic Diversity Studies

季维智　宿　兵　主编

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内容提要

本书是我国第一本系统介绍遗传多样性研究的原理与方法的专著。在遗传多样性保护的基本原理方面，主要介绍了遗传多样性的概念、迁地保护、异地保存、动物保护繁育和遗传管理，以及保护遗传学和分子生态学的原理、方法和进展；在遗传多样性保护研究的方法方面，具体介绍了样品的采集与提取、同工酶分析技术、DNA 限制性片段长度多态性分析、DNA 指纹图谱分析和随机扩增多态 DNA 的方法等。

本书读者对象为生物学和农林科学的研究人员、有关院校的师生和有关方面的管理人员。

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总　序

各种各样的生物资源是地球上人类赖以生存的基础。然而，由于人类活动的加剧，引起了全球环境的迅速恶化。最大限度地保护生物多样性已成为国际社会关注的热点。在 1992 年 6 月举行的联合国环境与发展大会上，包括中国在内的 153 个国家在《生物多样性公约》上签了字，从而使保护生物多样性成为世界范围内的联合行动。中国作为世界上生物多样性特别丰富的国家之一，不仅积极开展了生物多样性的保护活动，而且还最早制订了国家级生物多样性保护行动计划。

作为中国自然科学研究中心的中国科学院一直积极致力于生物多样性的研究工作。在国家科委、国家基金委等单位的支持下，经过四十多年的考察与研究，在许多课题和研究项目上取得了可喜的成绩，还先后组织编写了《中国植物志》、《中国动物志》、《中国孢子植物志》、《中国植被》、《中国高等植物图鉴》、《中国植物红皮书》(第一卷)等书，并增建和扩建了有关的研究设施如标本馆、植物园、定位研究站等，为中国生物多样性保护与持续利用提供了大量的资料和措施。为了加强生物多样性研究工作，在原生物多样性工作组的基础上，于 1992 年 3 月成立了中国科学院生物多样性委员会，统一协调生物多样性研究工作，并与国内外有关机构开展了各种形式的合作。

目前，中国科学院已有相当一批专家正在开展生物多样性方面的研究，从基因、物种、生态系统和景观四个水平上研究生物多样性的现状、受威胁或濒危的原因以

及保护与恢复的对策，并积极建设全国性的生物多样性信息系统，以期为中国的生物多样性保护与持续利用提供理论依据。

为了推动生物多样性研究工作，及时反映这方面的研究成果，促进跨世纪人才的培养，在继续编译《生物多样性译丛》的基础上，我们组织撰写了《生物多样性研究丛书》。这套丛书将集中介绍中国科学院生物多样性研究的最新成果和有关的基本原理与研究方法。由于生物多样性研究是综合性和实践性很强的新兴领域，编写这样的丛书也是我们的初步尝试，希望得到有关专家的积极支持，共同培育这棵刚刚破土而出的新苗。

许智宏

1996 年 9 月

序

遗传多样性是生物多样性的重要组成部分。广义地讲，遗传多样性就是生物所携带遗传信息的总和；狭义上，则指种内不同群体和个体间的遗传多态性的程度，或称遗传变异。遗传多样性是物种进化的本质，也是人类社会生存和发展的物质基础。"一个基因关系到一个国家的兴衰，一个物种影响一个国家的经济命脉"，已是被无数实例证明了的事实。如第一次"绿色革命"和水稻杂交优势的利用，就是发现和利用了矮秆基因和不育基因的结果。显而易见，遗传多样性的研究无论是对生物多样性的保护，还是对生物资源的可持续利用，以及未来世界的食物供应，都有重要的意义。

遗传学的研究在我国已有较长的时间，而遗传多样性的研究则是近几年的事，主要是在 1992 年中国科学院成立生物多样性委员会、积极推动生物多样性研究之后。遗传多样性研究领域是十分活跃的领域。一方面，生物多样性保护需要对物种或群体的遗传多样性进行深入的研究；另一方面，现代遗传学、保护生物学和分子生物学的理论、技术和方法的发展和应用促进了遗传多样性研究，随之产生了一些新的理论和学科。如分子生物学和生态学的有机结合形成了分子生态学；遗传学的原理和研究手段应用于遗传多样性的研究产生了保护遗传学。生物技术的发展和应用又大大拓宽了遗传多样性的保护途径。

作为我国这样一个生物多样性十分丰富的、同时也是人口众多的泱泱大国，遗传多样性的研究工作就更为

重要了。我们需要博取他人之长，尤其是新的理论、技术和方法，使我国的遗传多样性的研究适应社会、环境的需要。《遗传多样性研究的原理与方法》是一本既有基本原理阐述，又有具体实验操作方法，兼涵理论和实践于一体的好书。值得一提的是，本书的作者多为一线的科研人员，而且多为青年科技人员。本书既反应了当前遗传多样性的研究进展，也有作者在实际工作中的实践经验，这无疑对我国的科研、教学人员会有很大的帮助。看到这一领域近年来涌现出来的新生力量，我感到欣慰。相信在不远的将来，我国遗传多样性的研究必将迈上一个新的台阶。

中国科学院院士　李振声

1999 年 4 月

前　言

遗传多样性被认为是生物多样性的核心问题。蕴藏于物种内或种间的分子、细胞和个体水平的遗传变异，是遗传多样性的基础，也是物种保持进化潜能的基本条件，与生物多样性的形成、消失和发展休戚相关。遗传多样性的研究工作是生物多样性就地保护的基础，更是迁地保护计划的关键。遗传资源的保护和利用，不仅是生物多样性保护的关键因素，也是大农业持续发展的需要，关系到世界未来的食物供应问题。因而，遗传多样性的研究也是生物多样性研究中发展较快的领域。一方面，现代生物学的理论、技术和方法（尤其是分子生物学和生物技术）被广泛应用于遗传多样性的研究；另一方面，研究结果和新方法的应用又形成了一些新的理论和学科，同时也使遗传多样性保护的理论和方法得到了很大的发展。

我国是一个生物遗传多样性丰富的国家。无论是野生生物资源（动物、植物、微生物等）还是家养动物、栽培植物，都在世界上占有重要地位，是大自然宝贵的遗产。尽管我国遗传多样性的研究近年来已取得了长足的进展，但与国外相比，仍有较大差距，保护遗传多样性的任务相当迫切。为促进我国遗传多样性的研究和遗传资源保护的开展，在中国科学院生物多样性委员会的主持下，我们编写了这本书，从遗传多样性保护的理论和实践两方面作了具体的介绍，试图让读者在了解相关理论的同时，也能掌握较详细的操作方法。

本书分 14 章。前 6 章着重介绍了遗传多样性的基

本概念和有关新学科的理论，如保护遗传学、分子生态学及遗传多样性保护的理论，有动物保护、繁殖的理论和遗传管理，以及遗传多样性的迁地保护和低温保存的原理等。后8章为相应的技术及方法，从样品的采集、提取方法开始，介绍了同工酶、DNA的RFLP、RAPD、指纹图谱及DNA序列分析等技术和方法，以及生殖生物技术在遗传多样性保护中的应用等。来自中国科学院植物研究所、遗传研究所、昆明动物研究所，以及中国农业大学、云南农业大学的22位作者参加了本书的编写。初稿完成后，马克平、昝瑞光、陈海如和王文等先生对书稿作了审定，在此深表谢意。

遗传多样性的研究发展迅速，新的理论和方法不断产生、完善和发展。限于编者水平，本书难免有遗漏和错误之处，还望有识之士予以指正。作为一本理论和方法兼容的书，我们希望能对有关研究者或读者有所帮助。

季维智　宿　兵

1998年6月

Summary

Genetic diversity is probably the central issue in the biodiversity. A species, in a sense, is a unique gene pool. Therefore the species diversity on earth also means the diversity of gene. However, the diversity of gene goes far beyond the scope of species diversity. Genetic diversity is expressed at many different levels. Every species is made up of many individuals. Modern genetics has proved that except for the cases of parthenogenesis and identical twins, or the clone ones, no two individuals share the same genome. Secondly, according to taxonomy, some species, especially polytypic species can be subdivided into many subspecies. Many species actually contain hundreds, or even thousands of different genotypes. Genetic variance at infra-and intra-specific levels show the rich genetic diversity within and between subspecies, and within and between different population of the same species. Intra-specific genetic diversity, also known as genetic polymorphism, is expressed at different levels. The first expression of intra-specific genetic diversity is at the morphological level. The second expression is at the chromosomal level. When we talk about the stability of the karyotype of a species, i. e. the number, morphology, and behavior of the chromosomes, we are speaking in relative terms. Polymorphism of chromosomes within species is a common phenomenon.

Because of the increasing great pressure of population, forest destruction by slash and primitive cultivation has been increased, and wildlife habitat rapidly decreased. It causes the genetic diversity loss so quickly. The shrinking and vanishing of genetic diversity has a far-reaching harmful effect. First of all, genetic diversity is the basis for evolution and adaptation. The more diverse and various of the intra-specific genes are, the better the species will adapt to the enviroment, and the greater potential the species will have in its evolution. Biodiversity at the species level will also contribute to the maintenance of the diversity of the ecosystem and slow down the process of extinction caused by problems in adaptation and evolution. It is widely accepted that a lack of genetic diversity threatens the existence of species or population.

For genetic diversity conservation and study, several theories or subjects, e. g. conservation genetics, molecular ecology, the theory of conservation breeding, have been developed or improved.

Conservation genetics, a new concept, employs genetic theories and molecular techniques in studying biodiversity (especially genetic diversity) and its conservation. Since species live in form of population, in which genetic diversity changes dynamically due to evolutionary forces: genetic drift and natural selection, genetic drift will remove alleles of a gene pool in a random way, especially for small population, which are usually caused by founder effects or isolations. Natural selection will dramatically change the genetic structure of a population. Hence, in our conservation strategies, genetic management for conserving genetic diversity

should be considered as one of the key points in practice. In addition, molecular systematics which aimed to reconstruct evolutionary events in the past, is also found to be very informative and useful in evaluating dynamics of genetic diversity and revealing population process.

Genetic management is an important issue of conservation genetics, which is based on the theory of genetics and specially using molecular techniques for preserving animals. The first thing of genetic management is to investigate genetic diversity. So far, a lot of molecular techniques are available for detecting genetic diversity at the levels of chromosome, protein and DNA. The more information about the molecular techniques will be summarized later. In a long run, genetic diversity conservation is to remain the evolutionary potential of a species according to the following six general principles: 1) Keeping genotype and phenotype of raising animals. 2) Eschewing mating of skin animals. 3) Keeping genetic diversity of raising animals. 4) Not developing raising animals domestically. 5) Determining conservation units (ESU) by genetic data. 6) Drawing up measures according to financial condition, gene flow and genetic diversity among different populations.

Molecular ecology is a new subject for biodiversity conservation, which is in the sense of the relationship between the individuals/population of organisms and their environments with the view of molecular biology. It might be directed at the interface among molecular biology, ecology and population biology. In other words, molecular ecology is to use the principles of population and genetics to study ecology and population biology and assess the ecological risk of the release of genetically modified organisms (GMOs) by means of molecular biology techniques. Population genetics, ecological genetics and evolution genetics provide a sound basis for the formation of molecular biology. Current progress of molecular ecology focuses on the relationship between gene variation and the environment.

In the practice for genetic diversity conservation, national parks, natural protected areas and wildlife refuges are all effective ways in situ preservation, endangered wildlife breeding centers, zoos, and centers for conservation of rare livestock and poultry, botanical garden and arboretum are common practices in ex situ preservation. Frozen bank is also an effective method for saving the germ and microbial diversity.

The theory of conservation breeding is important for the animals breeding in captivity, which is based on the theory of genetics and logistics. A series of problems need to be concerned about or resolved in the breeding: 1) About the number: The number of individuals needed in a breeding population to avoid inbreeding varies enormously from species to species. It is difficult to get enough number of individuals for captive breeding, but "effective population" is needed. 2) Inbreeding depression: Inbreeding will easily cause the problems of animal reproduction. To avoid the inbreeding depression, it is very important to know the genetic background of the breeders. Geneticists use "inbreeding coefficient" to express the inbreeding relationship of the animals. 3) Genetic variation: Genetic variation, the resource of evolution, is the true connection between the ideas of Mendel and Darwin. Only by maintaining the genetic variation can the animal breeding population avoid the inbreeding depression. 4) Several roles needed to maintain the genetic variation: a) Founders. The founders, the breeders in beginning breeding, should ideally be as heterozygous as possible, and each one should be unrelated to all the others. This means the founding gene pool should be as large as possible; b) Rapid multiplication. The best way is to breed offsprings from the founders as many as possible in a short period so that the reproductive biotechnology can be very useful; c) Equalization. It is important that each of the founders produce the same number of descendants, including the equalization of the genetic contributions on the males and females; d) Ring the changes. It is the best way by using artificial insemination to get another founder gene from other breeder population to maximize the genetic variation; e) Input from the wild. Introducing one unrelated individual from the wild can increase the captive breeders' genetic variation, but it is difficult for the endangered species as they are already very rare in the field; f) Generation time. This means diversity is maximized for the longest if the time between generations is maximized; g) Studbooks. Studbooks are the raw materials of planned breeding, containing the essential basic data in standard form. 5) Specify species. Starting captive breeding program, it should be considered that which specie is urgent, including the situation of species, e. g. status of endangered or keystone species. 6) Species. In the breeding, we need to know what is a species. For the view of conservation breeding, it is important to distinguish subspecies, hybrids and other complications; 7) Organization and breeding plans. There are several international active organizations to help conservation breeding. The famous ones include IUCN, WWF, the International Union of the Director of Zoological Gardens (IUDZG), the Conservation Breeding Specialist Group (CBSG), EEP, and so on. It makes sense for different groups to perform different tasks.

Beside the above roles, the living conditions of animals in zoos should be carefully considered about. We should prepare diets, such as vitamins, mineral, and aliphatic acid, and carefully turn to the needs of particular groups of species and the different species.

On the other hand, animal reproductive biotechnology, artificial insemination, oocytes maturation and fertilization in vitro, embryo development in vitro and embryo transfer, nuclear transplantation, gene transfer and cloning animals are also ways to save wild animals.

In vitro fertilization (IVF) and embryo transfer (ET) are the two basic techniques of the reproductive biotechnology. IVF means taking the gametes (eggs and sperm) from the animals and culturing to maturation, and then fertilizing in vitro. The procedure of the IVF is similar in most of the mammals though some alteration is existed in the different species. The culture condition, especially medium, is the key factor for IVF, among which, water quality is a very important and basic factor. The goal of embryo transfer is the placement of embryos in the uterus with minimal trauma to both embryos and recipient. The details of IVF for rhesus monkey and bovine are described in Chapter 14.

Nuclear transplantation is the replacement of the one cell nuclear with that of another. The techniques have developed so rapidly since 1980's because some problems were mostly overcome, such as media improvement, micromanipulation carried out on the o. cyte and embryo. However, the successful rate for nuclear transplantation on the mammals is still low. The key problem concerning the donor nuclear doesn't develop synchronously with the recipient cytoplasm. The techniques for cattle have been described in the book.

Gene transfer is the introduction of exogenous gene to the zygotes at the stage when two primary nuclei are formed. DNA *microinjection* is the most wildely applied method for gene transfer in mammals. In recent years, embryonic stem (ES) cell transfer is another popular method. The gene transfer is currently used in laboratories and domestic animals are pioneer in using the mouse model. The mouse continues to serve as a starting point for implementing gene transfer procedures and it is the standard for optimizing experimental efficiency for other species.

Botanical garden and arboretum are major activities for plants conservation ex situ in both their research and education programs. Some difficulties for species transfer from one natural community to another are due to lack of knowledge about the ecology of any plant species. But to their immobility, plants have more practical advantages than animals on ex situ conservation. In general, the role of the issue contains: 1) distinguishing genetic form environmental effects by observing variation among the growing plants in garden plots; 2) samples, which were taken from a range of population, therefore, if genetic variability is to be adequately represented; 3) avoiding domestication.

Methods of conservation for plants can be classified according to the parts of the plant: one whole organism, seed, tissues, or genetic materials in culture. The technology, in general, includes division of the rootstock, cuttings, tissue culture, seed bank and clone. There are advantages and disadvantages for the technologies. For instance, keeping whole plants has values of educational and research on its reproductive maturity, mature specimens and so on, but it has higher maintenance cost and needs more space. Grown in single species plantations, the whole plants are more susceptible to communicable diseases, while storing seed is much better than keeping whole plants. The principal advantages of seed bank are the economy of space and the large sample sizes. The principal disadvantages of seed bank are their reliance on a dependable power supply, the need for meticulous monitoring of germ inability over time, and the need for period regeneration under conditions that minimized selection among the residual seed stock. Tissue culture can maintain genotypes unaltered for a long time, provide economic means of suspending, and change in gene frequency. However, the culture may be made chromosomal unstable over a long time the same as the current using media. DNA libraries are probably the most stable forms in which genetic information can be stored.

To save microorganisms is different from animals and plants, because microorganisms are not typically classed as plants or animals, which include bacteria, cyanobacteria, fungi, protozoa and virus, but they are important to humans for the benefits and harmful effects. Microorganisms are also the essential parts of environment, contributing to the maintenance of stable ecosystems because they are found in nearly all environments. Unfortunately, conserving the microbial diversity was not concerned as that for the animals and plants. However, existing conservation programs for animals and plants diversity, such as the nature reserves, will likely cover all but a few specialized environments, so it should not establish special reserves for maintaining microbial diversity. However, we also need to preserve microorganisms off site.

The techniques to maintain microbial (virus, bacteria, fungus) diversity include: 1) Isolating and sampling. These methods are used to obtain pure strain microorganisms for special needs and keep them in a suitable medium, which contains the nutrition, as the microorganisms need; 2) Microbial identification. It is important to identify the microorganism as you want before you start to culture it. The procedure of the identification involves staining standard and microscopic examination, and also includes biochemical analysis of proteins and DNA; 3) Storage of microorganisms. To preserve microorganisms is to maintain a strain for an indefinite period or continuous culture.

For all biological materials preservation, cryopreservation and freeze-drying are the preferred techniques for long-term storage. The potential of storing lives is extended to many thousands of organelle, cell, tissue, organ, and body types including microorganisms, plants and animals. More recently, cryopreservation has been used as a appropriate technique to preserve plants and animal species. However, many cells and tissues which need for long term bio-storage await suitable methodologies. As different biologies of organisms make different responses to cryoprotectants and freezing, preservation protocol may need adjustments, or be constructed afresh for the materials under study. The protocols of the techniques have been developed in the expert laboratory for different biological materials. Freezedrying is widely employed to conserve micro-biodiversity. This is one of the key roles performed by microbial culture collections. In chapter 6, 13 and 14, we compile the protocols which are reproducible, robust, and in most instances have been transferred quite successfully to other laboratories. However, there is no unique method to store all biological material.

Another very important issue for the study of genetic diversity is molecular biology. Advanced in molecular biology, especially advanced in recombinant DNA techniques great attentions have been attracted to the polymorphism of gene itself. So far, a lot of molecular techniques are available for detecting genetic diversity at the levels of chromosome, protein and DNA. Of all the techniques for detecting DNA polymorphism is the most developed and informativeone, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), DNA sequences, DNA fingerprinting, microsatellite DNA and isozyme analysis, which are very useful for the genetic diversity analysis at molecular level.

However, starting to study genetic diversity of animals and plants by molecular techniques, one of the key steps is to collect DNA samples from animals and plants. We describe some roles according to the previous reports and our experiences. In general, if we aim to do a good sampling and make the samples to be used as much as we can, several points should be kept: 1) Collecting animal and plant samples. It always needs to do some measurements before collecting samples, such as the classification of samples, asymmetrical analysis, genetic variation, and physiological examination (or records). Samples collected from plants and animals should be appropriately stored according to the needs of different studies. Usually, samples for chromosomal study had to be sterilized, and those for protein electrophoresis and