生物多样性研究丛书

中国动植物的遗传多样性

Genetic Diversity of Animals and Plants in China

胡志昂　张亚平　主编

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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

生物的遗传多样性是地球上所有生物的遗传信息的总和，是生物多样性的三个组成部分之一，是物种和生态系统多样性的重要基础。遗传多样性是指物种的种内或种间个体间的基因变化。

中国丰富多样的野生动植物和微生物是遗传多样性珍贵的宝库。正是这些丰富的遗传多样性为中国物种多样性的形成奠定了基础，并通过丰富的物种多样性形成了不同类型的生态系统。更由于中国特殊的古气候和古地理的综合作用，导致中国具有极为丰富的特有种，使遗传多样性的研究更具有特别的重要性。不仅如此，中国的栽培植物和驯养动物的遗传多样性也极为丰富，许多地方品种及其野生近缘种的研究，同样具有重要的理论意义，并将产生巨大的经济效益和社会效益。

随着分子生物学和生物技术的发展，遗传多样性的研究、遗传资源的保存及其持续利用越来越受到各国政府和公众的重视。由于各种原因，中国的遗传多样性研究启动较晚，90年代以前只有少数实验室做一些零星的实验。本世纪90年代初，中国科学院成立了“生物多样性委员会”，着手组织全院的生物多样性研究工作。“八五”期间，中国科学院实施了院重大项目——“生物多样性保护与持续利用的生物学基础”，组织了昆明动物研究所、植物研究所、遗传研究所、应用生态研究所的部分学者开展“野生动植物群体遗传多样性研究”课题。通过5年的努力，已经圆满地完成了课题预定的计划目标，取得了重大进展和丰硕成果。这些研究成果表明，我

国遗传多样性研究已全面与国际接轨，引起了国际同行们的关注。作为中国科学院重大项目的主持人之一，我很荣幸地为之作序。

陈灵芝

1997 年 4 月 9 日

前　言

遗传多样性是生物多样性的重要组成部分。生物的遗传信息储存于DNA分子中，DNA序列变化导致生物发生形态、细胞、生理、生物化学各方面的变异，也决定生物的生态特性。几十年来农林牧业品种遗传一致性造成疾病大流行和大爆发的教训反复说明了保护遗传多样性的重要性和迫切性，也暗示了遗传多样性的丰富程度决定了物种对环境变化的适应能力和进化潜力。

遗传多样性研究为系统学和生态学提供了各种遗传标记。分子标记的应用促进了分子进化研究、分子系统学和分子生态学的发展。遗传多样性研究也为动植物育种提供了遗传标记。近年来标记辅助育种大大加快了育种的步伐。利用紧密连锁的分子标记，用定位克隆技术可以克隆包括产量性状在内的各种有经济价值的基因。

我国遗传多样性基础研究长期得不到重视，动植物遗传多样性保护和利用得不到足够的理论指导。我国很重视遗传资源的收集，作物种质库、圃保存了极大量的作物及其野生近缘种的材料，但因为缺少群体遗传结构数据，取样保存和管理的效率就很成问题。我国农林业因种子混杂，每年造成巨额经济损失，种子纯度鉴定技术落后是其主要原因之一。对珍稀濒危物种的保护也因不了解群体的遗传结构而不能采取有效的措施，而且不能对已有措施的效率进行监测。

1991～1995年，中国科学院组织了“生物多样性保护与持续利用的生物学基础”等重大项目，其中包括中

国野生动植物遗传多样性的研究。5年期间，我们建立了各种检测DNA多样性的最新方法，如随机扩增多态DNA(RAPD)、DNA扩增指纹(DAF)、微卫星DNA、DNA序列分析及非损伤性DNA分析技术，说明我们已经全面与该领域的世界水平接轨。应用这些新方法，我们从遗传多样性的角度探讨物种濒危的原因，并提出了就地保护的措施和科学的迁地保护的遗传管理方法；根据各个基因序列的信息构建各自的分子系统树；通过"联合"或是"一致"的途径构建物种树；从系统进化上来确定"进化上具显著意义的单元"(Evolutionarily Significant Unit，简称ESU)作为生物保护的基本单元。植物遗传多样性研究侧重生态系统建群种和我国原产作物近缘野生种，应用和改进了新的检测DNA多样性的方法，证明了生物哲学的预言：有性繁殖生物个体遗传组成的唯一性(uniqueness)。

本书第1章讨论分子系统学和生物保护的基本单元ESU，第2至第18章按物种分章叙述，其中，第2至第10章是关于野生动物的遗传多样性和进化的，第11至第14章是关于家养动物的遗传多样性的，最后4章涉及野生植物群体的生态遗传学和基因资源的取样保存策略。

本书献给中国科学院院士施立明教授。

胡志昂　张亚平

1997年3月

Summary

Genetic diversity that is one of three basic components of biodiversity has been defined as the total summation of genetic information of animals, plants, micro-organisms all around the world. As every body knows, genetic information is stored in DNA molecules. Nucleotide sequence variation of DNA leads to genetic variation of organisms at all levels: morphological, cytological, physiological, biochemical and molecular ones. In a narrow sense, genetic diversity generally means genetic variation within populations and among populations of species. The topics in this book can be divided into three parts:

1. Genetic diversity, molecular evolution and conservation biology of rare and endangered wild animals in China (chapter 2 to chapter 10).

2. Genetic diversity of livestock with special reference to gene resources from southwestern China (chapter 11 to 14).

3. Genetic structure of natural populations for some plants those are very important ecologically and economically (chapter 15 to 18).

Firstly a general theoretical topic was discussed, that is:

Species Tree and "Evolutionarily Significant Unit": Approach From DNA Sequence Analysis

The recent progress in reconstruction of species tree and identification of "evolutionarily significant unit" by using DNA sequence analysis has been reviewed. It is much more difficult to extract evolution information from DNA sequence than to obtain DNA sequence itself. It is suggested that attention should be paid to the following points:

1. Different genes or regions evolve at different rate. Therefore, it is important to identify DNA fragments with reasonable evolutionary rate, so that we can detect more variations between taxa studied and reduce the background at the mean time. Slow evolved genes are good for comparison between distant related taxa, and fast evolved genes are good that between closely related taxa.

2. Correct alignment of the DNA sequences is essential for phylogenetic reconstruction. Any alignment obtained by computer software should be checked by eye.

3. There are two general approaches to obtain the overall estimate of the phylogeny from multiple gene sequences. First, the data sets can be combined from outset, with the phylogenetic analysis being performed on the combined data set—the combined approach. Alterna-

tively, phylogenetic trees can be estimated separately from each data set and a consensus tree determined from these trees—consensus approach. It appears that, by more directly utilizing the testimony of all the characters, a combined method may allow a closer approach to the true phylogeny, and a combined tree can be more resolved than a consensus tree.

However, if there is evidence on nonindependence of variations within or between the genes examined, consensus approach is preferred over combined approach.

4. Different genes or different sites in the same gene may subject to different selection pressure, and they evolve at different speed. Therefore, sequence weighting is necessary in the phylogenetic estimation. It is in general agreement that slower evolved regions and sites should be weighted higher than faster evolved regions and sites, and transversions should be weighted higher than transitions.

5. There are three major families of methods for inferring phylogeny: the parsimony and compatibility methods, the distance methods, and the maximum likelihood methods. No method allows one to make inference about evolutionary patterns in a well-justified way without making assumptions about evolutionary processes. However, the assumptions inherent in these methods are only sketchily known—we have hints but little in the way of comprehensive proofs that particular assumptions are required. Therefore, a useful exercise is to estimate phylogenetic trees with several different methods. The agreement among trees estimated by different methods lends greater credibility.

6. Gene tree and species tree are different in two aspects; (1)topologies may be different, and (2)branch length which represent divergence time may be different. However, if we combine data from many loci, it is possible to obtained a gene tree which is reasonably close to the species tree. In that case, we can take the gene tree as species tree.

7. The "Evolutionarily Significant Unit" (ESU) is increasingly accepted as the conservation unit. The criteria for recognizing an ESU was suggested by Moritz: ESUs should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci. A true phylogeny is essential for identification of ESUs.

From chapter 2 to 10, genetic diversity, molecular evolution and conservation biology for rare and endangered wild animals were discussed.

Genetic Diversity of the Giant Panda

The giant panda (*Ailuropoda melanoleuca*) is classified as endangered species by the Conservation on International Trade in Endangered Species (CITES), the International Union for the Conservation of Nature, and the governments of China and other countries and is the subject of controversy regarding conservation efforts. However, we almost know nothing about the giant panda natural population genetic structure and inbreeding coefficient, which is nec-

essary for the conservation efforts. Furthermore, there are many more captive giant pandas with unresolved parentage. Therefore, paternity determination is greatly needed for analyses of the number of founder genes present in the captive population, for assessments of the success of natural vs. artificial matings, and for other genealogical and population analyses. A non-invasive approach will make the genetic study on the freeranging population possible. The recent progress in our laboratory on the study of genetic diversity of the giant panda was summarized as follows:

1. Protein polymorphism. Twelve panda blood samples from Yuexi, Baoxing, Meigu, Miabian, Pingwu, Nanping, Maowen and Leibo have been collected. 17 Asiatic black bear blood samples from Xishuangbanna and Ruili of Yunnan Province were also collected for comparison. Thirty six kinds of blood proteins and isozymes encoded by 40 genetic loci by using standard starch gel electrophoresis were analyzed. Among all the loci, only Xanthine dehydrogenase-2 locus on the giant panda is polymorphic. The percentage of polymorphic loci (P) is 0.025 and the mean individual heterozygosity (H) is 0.008. In contrast, the black bear exhibits much higher levels of allelic diversity; P=0.216, and H=0.056. Our results indicate that the giant pandas are genetically monomorphic.

2. Mitochondrial DNA sequence variation. Forty samples which represent 21 founders: 2 from Mabian, 1 from Meigu, 2 from Yuexi, 11 from Baoxing, 1 from Pingwu, 2 from Qingchuan, 1 from Nanping, and 1 from Baishuijiang were collected. DNAs were extracted from liver, heart, blood or hair samples. A total of 336-444 bp of mitochondrial tRNA gene and D-loop fragment from each sample were amplified and sequenced. Nine haplotypes were detected in the giant panda populations. There are three and two sites characterized with transition and insertion/deletion, respectively. Parsimony analysis demonstrated that there was not significant genetic difference between different geographical populations. The genetic diversity level in the giant panda is much lower than that in any of the bear populations. The current populations occurred in late Pliocene. The results suggest that it is more reasonable to build corridors only between isolated populations within each mountain region in the conservation efforts.

3. Microsatellite DNA and paternity identification. We constructed DNA library of 150-500 bp fragments digested from giant panda genomic DNA with Dpn II constructed. Ten microsatellite DNA loci were isolated and characterized by screening the library with ^{32}P-labelled $(CA)_{15}$ probe. The specific primers for each of the microsatellite DNA locus were synthesized and employed to amplify DNA samples isolated from 7 specimens of panda tissue and 6 specimens of hair. Nine of the 10 microsatellite DNA loci were polymorphic in the 13 samples. Inheritance patterns of allele at these loci accurately agree with the well recorded pedigree. The polymorphism on these loci also clarified some previously unresolved paternity. These results imply the screened microsatellite DNA loci are useful markers for the identification of kinship among giant panda.

Genetic Diversity and Evolution of Genus *Muntiacus*

Genus *Muntiacus* is a widely concerned mammal group as a model for studies on cytogenetics and evolution. In this chapter, chromosome banding, chromosome painting and mitochondrial DNA RFLP were assayed to study this group. A male muntjac, whose taxonomic position is not confirmed yet, was captured from southwestern China, Gongshan County, Yunnan province in 1988. We prepared its miotic chromosome preparations by short term culture and routine air-drying method. The synaptonemal complex of spermatocytes are prepared by surface-spreading and silver-staining technique. The diploid chromosome number is found to be 9, and 8 in female will be expected, which are different from those of Indian muntjac, Fea's muntjac, Roosevelt's muntjac and Chinese muntjac. The No. 1 chromosome pair is heteromorphism, e. g. an homologue is X-autosome compound, the other is acrocentric, which is confirmed by SC analysis. Nos. 2, 3 are large submetacentric chromosomes. No. 4 is acrocentric. Y is a small chromosome. Two pairs of Ag-NORs are located on the long arm of Nos. 1, 4 chromosomes, which are quite different from those of the black muntjac. C-bands are found at centromeric region of all chromosomes and the "neck" region of X-autosome compound have abundant heterochromatin. The results suggest that the Gongshan muntjac might be separated from existing five species and support its identification as a new species. We have used a combination of chromosome sorting, degerated oligonucleotide-primered polymerase chain reaction (DOP-PCR), chromosome painting and digital image capturing and processing techniques for comparative analysis of members of the genus *Muntiacus*. Chromosome-specific "paints" from a female Indian muntjac were hybridised to the metaphase chromosomes of the Gongshan, Black and Chinese muntjac by both single and three color chromosome painting. Karyotypes and idiograms for the four muntjacs were constructed, based on enhanced 4', 6-diamidino-2-phenylindole (DAPI) banding patterns. The hybridization signal for each paint was assigned to specific bands or chromosomes for all of the above muntjac species. The interspecific chromosomal homology was demonstrated by the use of both enhanced DAPI banding and comparative chromosome painting. These results provide direct molecular cytogenetic evidence for the tandem fusion theory of the chromosome evolution of muntjac species. The mitochondrial DNA restriction maps for 12 restriction enzymes of four species of muntjacs: Indian muntjac (*M. muntjak*), Gongshan muntjac (*M. gongshanensis*), black muntjac (*M. crinifrons*), and Chinese muntjac (*M. reevesi*), were compared to estimate the phylogenetic relationships among them. Phylogenetic trees were constructed by both distance and parsimony method. The two resulted trees share similar topology which indicate that the black muntjac and the Gongshan muntjac are closely related followed by the Chinese muntjac, the Indian muntjac is the sister taxon to all the other muntjacs.

Molecular Evolution and Genetic Diversity of Arctoidea

1. mtDNA sequence variation. Sun bear is found in Burma, Thailand, Vietnam, Sumatra, Borneo, North-east of India, Malaysia, and Yunnan and Sichuan of China and has been listed in 1990 IUCN Red List of Threatened Animals as a vulnerable species. In this chapter, a total of 1174 bases of mitochondrial DNA sequence (397 bases of cytochrome b gene, 346 bases of 12S rRNA gene, 98 bases of tRNA genes, and 333 bases of D-loop region) from each of four sun bears from different collections have been sequenced. Comparing with the black rhino tRNA genes, our results suggest that the loop regions of tRNA genes evolve much fast than the stem regions. In the cytochrome b gene, 12S rRNA gene and tRNA gene regions, no sequence variation among the individuals was observed, which indicates that genetic variation in the sun bear is relatively low. In the D-loop region, there are 13 and 1 sites, respectively, characterized by transition and transversion among the 4 sun bears sequenced. The relationships among all the individuals were fully resolved based on mitochondrial D-loop sequences by using parsimony analysis. Our results suggest that the mitochondrial D-loop region is very useful for population genetic study of the sun bear.

2. Molecular evolution. The phylogenetic relationships among some bear species are still open questions. We present here mitochondrial DNA sequences of D-loop region, cytochrome b, 12S rRNA, $tRNA^{pro}$, and $tRNA^{Thr}$ genes from all bear species and the giant panda. A series of evolutionary trees with concordant topology has been derived based on the combined data set of all of the mitochondrial DNA sequences, which may have resolved the evolutionary relationships of all bear species: the ancestor of the spectacled bear diverged first, followed by the sloth bear; the brown bear and polar bear are sister taxa relative to the Asiatic black bear; the closest relative of the American black bear is the sun bear. Primers for forensic identification of the giant panda and bears are proposed. Analysis of these data, in combination with data from primates and antelopes, suggests that relative substitutional rates between different mitochondrial DNA regions may vary greatly among different taxa of the vertebrates.

3. Protein polymorphism. We examined protein polymorphism of Asiatic Black Bear from two areas of Yunnan Province, China. Forty genetic loci were analyzed. The result showed that the percentage of polymorphism loci (P) is 0. 216, the mean hetreozygosity (H) is 0. 056. It indicates that the Asiatic populations are weathly of genetic diversity.

4. Phylogenetic relationship. The Arctoidea is a superfamily of Carnivora that contains eight families: the bears (Ursidae), giant panda (Ailuropodidae), raccoons and allies (Procyonidae), lesser panda (Ailuridae), skunks, otters, weasels, and relatives (Mustelidae), sealions and eared seals (Otariidae), seals (Phocidae), and the walrus (Odobenidae). The phylogenetic

relationships among many families are very controversial. For example, based on parsimonious analysis of morphological characters, Wyss and Flynn (1993) suggested that the pinnipeds were monophyletic and related to bears: (Mustelidae, (Procyonidae, (Ailuridae, (Ursidae, (Otariidae, (Phocidae, Odobenidae)))))). However, also based on morphological characters Wozencraft (1989) suggested that the pinnipeds were diphyletic: ((((Otariidae, Odobenidae), (Ailutidae, Ursidae)), (Canidae, (Procyonidae, (Mustelidae, Phocidae)))). Using highly repetitive DNA, Arnason and Widegren (1986) showed that that the pinnipeds were monophyletic and related to the Mustelidae. To examine different hypotheses on the phylogeny of the Arctoidea, we sequenced segments of mitochondrial cytochrome b and 12S rRNA genes from the Southern sea lion, bears, giant panda, lesser panda, raccoon, kinkajou, coatimundi, black-footed ferret, and the hunting dog. Both the cytochrome b and 12S rRNA genes show a strong mutational transition bias. Parsimonious analysis based on the transversions of 12S rRNA gene, and substitutions at the first and second codon positions and transversions at the third codon position of the cytochrome b gene supports the placement of the Procyonidae with the Mustelidae, and the Ailuropodidae with the Ursidae (Wozencraft, 1989). Our findings indicated that the Otariidae share a common ancestor with the Procyonidae and Mustelidae rather than with the Ursidae. Interestingly, according to the current viewpoint of diphyletic origin of pinnipeds, the Phocidae shares a common ancestor with the Mustelidae whereas the Otariidae and Pdobenidae share a common ancestor with the Mustelidae whereas the Otariidae and Odobenidae shares a ancestor with the Ursidae. Therefore, our results do not support the diphylrtic origin of the pinnipeds. Our findings suggest that the Ailuridae is not closely related to either the Ursidae or the Procyonidae, and may represent an early radiation with the Arctoidea. Further investigation on more representative species from each families will be required.

Molecular Evolution and Genetic Polymorphism of Golden Monkey

1. Studies on cytogenetics. A systematical analysis of chromosome characteristics were carried out between Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) and Sichuan snub-nosed monkeys (*Rhinopithecus roxellanae*). G-band and Ag-NOR showed that there is no significant difference between them. But significant difference exist in C-band.

2. Protein polymorphism. We examined protein polymorphism of Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) from two areas of Yunnan Province, China. Fifty two genetic loci were analyzed, only one polymorphic locus was observed. Therefore, the percentage of polymorphic loci (**P**) is 0.019, the mean heterozygosity (**H**) is 0.005 and the mean number of alleles (**A**) is 1.019. Our results indicate that the *R. bieti* may be depauperate in genetic diversity at the level of proteins. We infer that *R. bieti* experienced a population bottleneck

in history, and the unavoidable inbreeding afterward resulted in the paucity of allelic diversity, which may be a leading reason for its present endangered situation.

3. Mitochondrial DNA sequence variation. The classification and phylogenetic relationships of the snub-nosed langurs (*Rhinopithecus*) are still open questions. We have sequenced a mitochondrial cytochrome b gene fragment from *R. roxellana*, *R. bieti*, *R. avunculus* and *Presbytis phayrei*. There are 47 sites (19%) characterized by variation. A series of evolutionary trees with concordant topology has been derived by using parsimony, maximum likelihood and distance methods, which may have resolved the evolutionary relationships of the three golden monkey species. *R. bieti* is more closely related to *R. avunculus* than to *R. roxellana*. The divergence among those three species occurred about 2—6 million years ago. Our results suggest that *Rhinopithecus* is a valid genus, and *avunculus* should be placed into this genus. Our noninvasive genetic analysis of a captive population revealed low genetic diversity of *R. bieti*.

4. RFLP of ribosomal DNA. Restriction maps of nontranscribed spacer of ribosomal DNAs from five species of Colobinae and three outgroup taxa, *Hylobates Leucogenys*, *Macaca mulatta*, *and M. irus*, were constructed using 15 different restriction endonucleases and cloned human 18S and 28S rDNA probes. The site difference between *Rhinopithecus roxellanae and R. bieti* is comparable to that between *Presbytis francoisi* and *P. phayrei*, implying that *R. bieti* is a valid species rather than a subspecies of *R. roxellanae* as classified by Groves (1970). Phylogenetic analysis of the data supports *Rhinopithecus* should be an independent genus, since it has distinct distance to genus *Pygathrix* as well as *Presbytis* on the phylogenetic tree. *Pygathrix* represented by *P. nemaeus* is closely related to the *Presbytis* rather than *Rhinopithecus*. The branching pattern and branch length among different nodes support the hypothesis that the leaf monkey is an intermediate taxon between old world monkey and gibbon.

5. RAPD. We analyse Random Amplified Polymorphism DNA (RAPD) and genetic polymorphism of six capitive breed Yunnan snub-nosed monkeys (*Rhinopithecus bieti*). Forty-five 10bp short primers were used to amplify genomic DNA of *R. bieti*. About 130 RAPD markers were observed in each *R. bieti* and 80% RAPD marders showed no polymorphism. The genetic distance between *R. bieti* is 0.052. It indicates that the genetic polymorphism is very low in population of *R. bieti*. We also construct the pedigree of the six *R. bieti* by analysis of genetic distance and put forward a captive breed plan.

Genetic Diversity and Molecular Phylogeny of Slow Loris (Genus *Nycticebus*)

Chromosome studies, protein electrophoresis and mitochondrial DNA RFLP were used to demonstrate genetic diversity and phylogeny of genus *Nycticebus*. The karyotypes of three

species (*N. coucang*, *N. intermedius*, *and N. pygmaeus*) of genus *Nycticebus*, collected from southern Yunnan of China, have been surveyed. All individuals from three species possess 2n =50 chromosomes, and all chromosomes in their complement are biarm chromosome. The karyotype of slow loris (*N. coucang*) is characterized by having a secondary constriction and Ag-NORs on the short arms of pair No. 1. The G-banding patterns of three species are very similar. Three species are found to have multiple Ag-NORs. In *N. coucang*, NORs were observed on five pairs (Nos. 1, 6, 9, 15, and 23) and in N. Intermedius and *N. pygmaeus*, NORs were found on four pairs (Nos. 6, 9, 15, and 20). This finding indicates that slow lorises, as primitive primates, also have multiple NOR-bearing chromosomes. The chromosome studies imply at least two valid species, *N. coucang* and *N. pygmaeus*. We examined protein polymorphism of 29 slow lorises (*N. pygmaeus*, 27, *N. coucang*, 2), which derived from south-western Yunnan and northern Vietnam. Forty two genetic loci were screened, four were found to be polymorphic in *N. pygmaeus*. The percentage of polymorphic loci (**P**) is 0. 095, the mean individual heterozygosity (**H**) is 0. 040 and the mean number of alleles (**A**) is 1. 045. Furthermore, we calculated the genetic distance (D) between the two species, D=0. 2541, which indicate a valid species status of each. For mtDNA analysis, eight restriction types were observed in the three suggested species. Phylogenetic trees constructed on the basis of genetic distances showed that the slow lorises sort into two clusters: four types of *N. coucang* and three types of *N. intermedius* plus one type of *N. pygmaeus*. The mtDNA results suggest that three are two valid species in the genus *Nycticebus*, *N. coucang* and *N. pygmaeus*, and that *N. intermedius* should be included within *N. pygmaeus*. Divergence between the two species may have begun 2. 7 million years ago. Evolution of gross morphology, chromosomes, protein electrophoresis and mitochondrial DNA in the slow lorises appears to be concordant.

Molecular Evolution and Genetic Diversity of Chinese Macaques (*Macaca*)

1. The spermatocyte synaptonemal complex karyotypes of four macaque species. With a combination of detergent-microspreading and silver-staining techniques, the spermatocyte synaptonemal complexes (SCs) of 4 species of macaques i.e. rhesus macaque (*M. mulatta*), pigtail macaque (*M. nemestrina*), Assam macaque (*M. assamensis*), Tibetan stumptailed macaque (*m. thibetana*) and a subspecies of rhesus monkey (*M. m. lasiota*) were observed by electron microscope. The results demonstrate the high similarity of SC karyotypes as well as its development among these macaques. The autosome synapsis or pairing of autosomal lateral elements starts at early aygotene, completes at pachytene and disppears at diplotene. Five types of XY have been described, on the basis of patterns of XY pair. The morphology of XY axes and homology of X and Y were also discussed.