

Molecular evolution study in China: progress and future promise

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China has a large land area with highly diverse topography, climate and vegetation, and animal resources and is ranked eighth in the world and first in the Northern Hemisphere on richness of biodiversity. Even though little work on molecular evolution had been reported a decade ago, studies on both the evolution of macromolecules and the molecular phylogeny have become active in China in recent years. This review highlights some of the interesting and important developments in molecular evolution study in China. Chinese scientists have made significant contribution on the methods inferring phylogeny and biogeography of animals and plants in East Asia using molecular data. Studies on population and conservation genetics of animals and plants, such as Golden monkey and Chinese sturgeon, provided useful information for conserving the endangered species. East and South Asia has been demonstrated to be one of the centres of domestication. Origin and evolution of genes and gene families have been explored, which shed new insight on the genetic mechanism of adaptation. In the genomic era, Chinese researchers also made a transition from single-gene to a genomic investigation approach. Considering the fact that amazing progress has been made in the past few years, and more and more talented young scientists are entering field, the future of molecular evolution study in China holds much promise.

Keywords: molecular evolution; China; phylogeny; biogeography; conservation genetics; gene

1. INTRODUCTION

China has a large land area with highly diverse topography, climate and vegetation, and a wealth of animal and plant resources. China is ranked eighth in the world and first in the Northern Hemisphere on richness of biodiversity (Zhang 1997). China is the home for approximately 10% of the world's biodiversity (Wu *et al.* 2004), which provides rich resources for studying molecular evolution. Even though there has been a strong research community in China in documenting the flora and the fauna, and remarkable achievements have been made over the past decades, little was done on the study of molecular evolution in China until a decade ago. Fortunately, the situation has been totally changed in recent years. The molecular evolution studies on the evolution of macromolecules and the molecular phylogeny became active and drew much international attention. For example, Chinese scientists contributed 26 papers in two important molecular evolution journals: *Molecular Biology and Evolution* and *Molecular Phylogenetics and Evolution* in 2004. We shall mention four key senior scientists, Professors Jiazhen Tan (C. C. Tan), Li-ming Shi, Yu-yi Chen and De-yuan Hong, who have made much effort

to promote molecular evolution studies in China. In 1990, the Key Laboratory of Cellular and Molecular Evolution was founded by Professor Li-ming Shi at Kunming Institute of Zoology, the Chinese Academy of Sciences. Since then, Kunming Institute of Zoology became the research centre in the area of molecular evolution in China. Such a key laboratory system is valuable to promote new disciplines in China. Here, we have made no attempt to cover all progress in molecular evolution, rather to consider interesting and important developments in China during the recent decade.

2. MOLECULAR PHYLOGENY

During the past 20 years, molecular phylogenetics has dramatically reshaped our views of organism relationships and evolution at all taxonomic levels of the hierarchy of life (Soltis & Soltis 2000). This trend has been manifested from recent studies on many groups in China.

Chinese scientists have made useful contributions on the methods inferring phylogeny. Both maximum-likelihood and maximum-parsimony (MP) methods play key roles for inferring phylogeny from DNA sequences. Felsenstein's (1981) maximum-likelihood method assumes that the rate of nucleotide substitution is constant over different nucleotide sites. This assumption is sometimes unrealistic. To solve this problem, Yang (1993) extended Felsenstein's method

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to the case where substitution rates over sites are described by the gamma distribution. He presented a numerical example to show that the method fits the data better than did previous models (Yang 1993). Yang (1996) investigated the assumptions underlying the MP method by studying the way the method works. He found that parsimony involves very stringent assumptions concerning the process of sequence evolution, such as constancy of substitution rates between nucleotides, constancy of rates across nucleotide sites and equal branch lengths in the tree. When a simple model of nucleotide substitution was assumed to generate data, the probability that MP recovers the true topology could be as high as, or even higher than, that of the likelihood method. When the assumed model became more complex and realistic, the probability that MP recovers the true topology, and especially its performance relative to that of the likelihood method, generally deteriorates. The likelihood method appears preferable to parsimony (Yang 1996).

Restriction fragment length polymorphism of mitochondrial DNA (mtDNA) was used to study phylogenetic relationships of the Old World monkeys (OWMs) and two panda at the early stage (Zhang & Shi 1989, 1991). DNA sequencing became the dominant technique in recent years, as DNA sequences can provide most abundant information for phylogenetic analysis. Even though mtDNA is still the most popular genetic marker and has been widely used for inferring phylogeny of animals, new nuclear markers have also been explored in recent years (Yu *et al.* 2004b; Yu & Zhang 2005). More nuclear genes are expected to be used in the near future. In addition, combined datasets of multiple genes can be useful to resolve some ambiguous phylogeny (Murphy *et al.* 2001; Yu & Zhang 2005, 2006). A similar trend has been observed in the studies on plants. Knowledge of the relationships among animal and plant groups is essential to understand the history of life, but also relevant to many studies of molecular evolution and developmental genetics.

3. MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF ANIMALS

As a biodiversity hot spot, China is an important region to understand origin and dispersal of many animals. There are over 3500 species of vertebrates and vascular plants in China. Phylogenetic analysis at the molecular level has shed some light on understanding the pattern of such biodiversity and the process involved.

The mammalian order Carnivora includes 11 families and classically has been divided into two monophyletic superfamilies, Caniformia and Feliformia. Despite numerous efforts, however, evolutionary relationships within and among the diverse families of living carnivores remain controversial (Wozencraft 1989; Zhang & Ryder 1993; Pecon Slattery & O'Brien 1995; Flynn & Nedbal 1998). Yu *et al.* (2004b) investigated the phylogenetic relationships among 37 living species of order Carnivora using nuclear sequence data from exon 1 of the interphotoreceptor retinoid-binding protein gene and first intron of the transthyretin gene. Their results strongly supported the

red panda as the closest lineage to procyonid–mustelid (i.e. Musteloidea) clade followed by pinnipeds (Otaridae and Phocidae), Ursidae (including the giant panda) and Canidae, the most recently diverged clade in the superfamily Caniformia. Four feliform families, namely the monophyletic Herpestidae, Hyaenidae and Felidae, and paraphyletic Viverridae, were consistently recovered convincingly. Yu *et al.* (2004a) analysed the phylogenetic relationships of all the seven bear species with seven genes. Their results supported basal position of the spectacled bear and sister-taxa association of the brown and polar bear, of Asiatic and American black bears, and of the sloth bear and sun bear, respectively. However, additional DNA sequences from more nuclear genes were needed to fully resolve the phylogeny of this family. In a further analysis of Caniformia using one mitochondrial and four nuclear genes (4417 bp), Yu & Zhang (2006) provided additional evidence to support the phylogenetic relationships recovered previously. To assess the evolutionary history of pantherine lineage of the cat family Felidae, six mitochondrial genes and three nuclear genes (≈ 6500 bp) were analysed. The monophyletic feature of the *Panthera* genus in pantherine lineage was confirmed and interspecific affinities within this genus revealed a novel branching pattern, with *Panthera tigris* diverging first in the *Panthera* genus, followed by *P. onca*, *P. leo* and the last two sister species *P. pardus* and *P. uncia*. In addition, the close association of *Neofelis nebulosa* to *Panthera*, the phylogenetic redefinition of *Otocolobus manul* within the domestic cat group and the relatedness of *Acinonyx jubatus* and *Puma concolor* were all important findings in the resulting phylogenies (Yu & Zhang 2005). The evolutionary relationships in the genus *Macaca*, the most widely distributed non-human primate, are still under debate. Li & Zhang (2005) sequenced five mitochondrial gene fragments from 40 individuals of 8 species: *Macaca mulatta*; *M. cyclopis*; *M. fascicularis*; *M. arctoides*; *M. assamensis*; *M. thibetana*; *M. silemus* and *M. leonina*. Combining with the data from *M. sylvanus*, they constructed phylogenetic trees using MP and Bayesian methods. Their results agree with earlier studies, suggesting that the mitochondrial lineages of *M. arctoides* share a close evolutionary relationship with the mitochondrial lineages of *fascicularis* group macaques (and *M. fascicularis*, specifically). *M. mulatta* (with respect to *M. cyclopis*), *M. assamensis assamensis* (with respect to *M. thibetana*) and *M. leonina* (with respect to *M. silemus*) are paraphyletic. Those studies demonstrated the power of an approach using multiple genes.

Molecular phylogenetic analysis is not only powerful to resolve the phylogeny relationships, but also useful to assess biogeographic scenarios underlying the geographical structure of lineages, to explore the factors shaping the geographical structure and to understand adaptation of animals.

Yu *et al.* (2000) investigated the molecular phylogenetic relationships among worldwide species of *Ochotona*. Their results strongly indicated three major clusters: the shrub–steppe group; the northern group; and the mountain group. They suggested that the differentiation of this genus in the Palaearctic Region was closely related to the gradual uplifting of the Tibet

(Qinghai–Xizang) Plateau, and that vicariance might have played a major role in the differentiation of this genus on the Plateau. On the other hand, the North American species, *Ochotona princeps*, is most probably a dispersal event, which might have happened during the Pliocene through the opening of the Bering Strait. These findings support the hypothesis that pikas have entered the steppe environment several times and that morphological similarities within steppe dwellers were due to convergent evolution (Yu *et al.* 2000). The phylogenetic analysis of musk deer based on the complete mitochondrial cytochrome *b* gene sequence supported the historic dispersion of musk deer in China from north to south, and Himalayan and the Hengduan Mountain areas in southwestern China as a divergence centre for musk deer (Su *et al.* 1999).

Oriental voles of the genus *Ochotona* are predominantly distributed along the southeastern shoulder of the Qinghai–Tibetan Plateau. Luo *et al.* (2004) investigated phylogeny of this genus based on mitochondrial gene sequences to test whether vicariance could explain the observed high species diversity in this area by correlating the estimated divergence times to species distribution patterns and the corresponding palaeogeographic events. Their results suggest that: (i) the eight species of Oriental voles form a monophyletic group with two distinct clades and these two clades should be considered as valid subgenera—*Eothenomys* and *Antelionomys*, (ii) Japanese red-backed voles are more closely related to the genus *Clethrionomys* than to continental Asian *Eothenomys* taxa, (iii) the genus *Clethrionomys*, as presently defined, is paraphyletic, and (iv) the process of speciation of Oriental voles appears to be related to the Trans-Himalayan formation via three recent uplift events of the Qinghai–Tibetan Plateau within the last 3.6 Myr, as well as to the effects of the Mid-Quaternary ice age.

The genus *Lepus* is monophyletic with three unique species groups: North American; Eurasian; and African. Ancestral area analysis indicated that ancestral *Lepus* arose in North America, and then dispersed into Eurasia via the Bering Land Bridge eventually extending to Africa. Brooks parsimony analysis showed that dispersal events, followed by subsequent speciation, have occurred in other geographical areas as well and resulted in the rapid radiation and speciation of *Lepus*. The genus appears to have arisen *ca* 10.76 Myr ago (± 0.86 Myr ago), with most speciation events occurring during the Pliocene epoch ($5.65 \pm 1.15 \sim 1.12 \pm 0.47$ Myr ago) (Wu *et al.* 2005).

The phylogenetic analysis of the subfamily Xenocyprinae from China suggested that species of *Xenocypris* and *Plagiognathops* form a monophyletic group that is sister to the genera *Distoechodon* and *Pseudobrama* (Xiao *et al.* 2001). Xiao *et al.* found that the introgressive hybridization might occur among the populations of *Xenocypris argentea* and *X. davidi*. The spatial distributions of mtDNA lineages among populations of *Xenocypris* were compatible with the major geographical region, which indicated that the relationship between Hubei+Hunan and Fujian is closer than that between Hubei+Hunan and Sichuan. From a perspective of parasite investigation, their data suggested that the fauna of *Hexamita* in Xenocyprinae

could be used to infer the phylogeny of their hosts (Xiao *et al.* 2001). The family Sisoridae is one of the largest and most diverse Asiatic catfish families, most species occurring in the water systems of the Qinghai–Tibetan Plateau and East Himalayas. Gao *et al.* (2005) used mitochondrial cytochrome *b* and 16S rRNA gene sequences to clarify existing gaps in phylogenetics and to test conflicting vicariant and dispersal biogeographic hypotheses of Chinese sisorids using dispersal–vicariance analysis and weighted ancestral area analysis in combination with palaeogeographic data as well as molecular clock calibration. Their results suggest that: (i) Chinese sisorid catfishes form a monophyletic group with two distinct clades; (ii) the glyptosternoid is a monophyletic group and *Glyptosternum*, *Glaridoglanis* and *Exostoma* are three basal species having a primitive position among them; (iii) a hypothesis referring to *Pseudecheneis* as the sister group of the glyptosternoid was supported; (iv) the genus *Pareuchiloglanis* is not monophyletic; (v) the uplift of Qinghai–Tibetan Plateau played a primary role in the speciation and radiation of the Chinese sisorids; and (vi) an evolutionary scenario combining the aspects of both vicariance and dispersal theory is necessary to explain the distribution pattern of the glyptosternoids. They tentatively estimated that the glyptosternoids most possibly originated in the Oligocene–Miocene boundary (19–24 Myr ago) and radiated from the Miocene to Pleistocene, along with a centre of origin in the Irrawaddy–Tsangpo drainages and several rapid speciations in a relatively short time.

More than 10 species within the freshwater fish genus *Sinocyclocheilus* adapt to caves and show different degrees of degeneration of eyes and pigmentation. Therefore, this genus can be useful to study evolutionary developmental mechanisms and the role of natural selection and adaptation in cave animals. To better understand these processes, Xiao *et al.* (2005) investigated the phylogenetic relationships of 31 recognized species based on the complete mitochondrial cytochrome *b* gene (1140 bp) and partial NADH dehydrogenase subunit 4 (ND4) gene (1032 bp). Their phylogenetic results showed that all species except for two surface species, *Sinocyclocheilus jii* and *S. macrolepis*, clustered as five major monophyletic clades (I, II, III, IV and V) with strong supports. *S. jii* was the most basal species in all the analyses, but the position of *S. macrolepis* was not resolved. The cave species were polyphyletic and occurred in these five major clades. The results indicate that adaptation to cave environments has occurred multiple times during the evolutionary history of *Sinocyclocheilus*. The branching orders among the clades I, II, III and IV were not resolved, and this might be due to early rapid radiation in *Sinocyclocheilus*. All species distributed in Yunnan except for *S. rhinoceros* and *S. hyalinus* formed a strongly supported monophyletic group (clade V), probably reflecting their common origin. Such results suggested that the diversification of *Sinocyclocheilus* in Yunnan may correlate with the uplifting of Yunnan Plateau.

The sand lizards of the genus *Phrynocephalus* (Family Agamidae; Kaup 1825) distributed from northern–western China to Turkey, and are one of the major

components of the Central Asian desert fauna. Pang *et al.* (2003) investigated the phylogenetic relationships among most Chinese species of lizards in the genus *Phrynocephalus* using four mitochondrial gene fragments (12S rRNA, 16S rRNA, cytochrome *b* and ND4-tRNA^{LEU}). Their results showed that there are two major clades representing Chinese *Phrynocephalus* species: the viviparous group and the oviparous group. All the analyses left the nodes for the oviparous group, the most basal clade within the oviparous group and *Phrynocephalus mystaceus* unresolved. The phylogenies further suggest that the monophyly of the viviparous species might have resulted from vicariance, while recent dispersal might have been important in generating the pattern of variation among the oviparous species.

Chen *et al.* (2004) reconstructed the phylogeny of the family Eurytomidae based on nuclear 28S and 18S rRNA genes, and mitochondrial 16S rRNA and cytochrome oxidase I genes. Their analysis revealed a significant incongruence between the mitochondrial and the nuclear genes. Their choice of results from the nuclear genes as their preferred hypothesis suggested that the family Eurytomidae was not a monophyletic group; neither were the genera *Eurytoma* and *Bruchophagus*. The monophyly of genera *Sycophila* and *Plutarchia* was well supported, as was the close association of the genera *Aiolomorphus*, *Tenuipetiolus*, *Bephratelloides* and *Phylloxeroxenus*. Their phylogeny also revealed an anticipated pattern in which species groups from the genera *Eurytoma* and *Bruchophagus* were often more closely related to other small genera than to other species groups of the same genus. However, more sequence data from both nuclear and mitochondrial genes are required to test their hypothesis. Furthermore, this family can be useful to explore the factors resulting in the incongruence between the mitochondrial and the nuclear genes.

4. MOLECULAR PHYLOGENY OF PLANTS

Recent advances in phylogenetic methodologies, together with the availability of new types of data (in particular, the DNA sequences), have significantly increased the accuracy of phylogenetic reconstruction of plants.

Pinaceae is the largest extant family of gymnosperms comprising 11 genera and more than 200 species (Farjon 1990). Many species of the pine family constitute the major forest elements in the northern temperate region. Owing to morphological convergence within the family, Pinaceae has been a phylogenetically complex group (Farjon 1990). Using sequences of the chloroplast *matK* gene, the mitochondrial *nad5* gene and the low-copy nuclear gene 4CL, Wang *et al.* (2000) studied intergeneric relationships of all the extant genera in Pinaceae. A combined analysis of the three gene sequences generated a well-resolved and strongly supported phylogeny that agrees to a certain extent with the previous phylogenetic hypotheses based on morphological, anatomical and immunological data. Disagreement between the previous hypotheses and the three-genome phylogeny suggested that morphology of both vegetative and reproductive organs has undergone convergent evolution within the pine family.

The strongly supported monophyly of *Nothotsuga longibracteata*, *Tsuga mertensiana* and *Tsuga canadensis* on all the three gene phylogenies provided evidence against the previous hypotheses of intergeneric hybrid origins of *N. longibracteata* and *T. mertensiana*. This study with a complete generic sampling of the family provided important insights into the phylogenetic relationships within the family by examining congruence and incongruence of gene phylogenies among the three genomes.

Chen *et al.* (1999) presented a comprehensive analysis of the phylogenetics and the systematics of the family Betulaceae from very diverse perspectives, including molecular, morphological and fossil evidence. They used *rbcL*, internal transcribed spacer (ITS) and morphological data to assess the phylogeny of Betulaceae, which was found to be monophyletic, with Casuarinaceae as its sister group. Within Betulaceae, two sister clades were evident, corresponding to the two traditional subfamilies Betuloideae (*Alnus* and *Betula*) and Coryloideae (*Corylus*, *Ostryopsis*, *Carpinus* and *Ostrya*). The molecular phylogenetic relationships among the extant genera were compatible with inferences from ecological evolution and the extensive fossil record (Chen *et al.* 1999).

As an important group of the subclass Hamamelidae of angiosperms, Fagales comprise eight families: Betulaceae, Casuarinaceae, Fagaceae, Juglandaceae, Myricaceae, Nothofagaceae, Rhoipteleaceae and Tico-dendraceae. Although the concept of the broadly defined order has received strong support from molecular systematic studies and its monophyly has been well established (see the reviews by Li *et al.* 2004), interfamilial relationships have not been resolved with certainty. Li *et al.* (2004) sequenced six regions from three plant genomes (the plastid *trnL-F*, *matK*, *rbcL* and *atpB*; the mitochondrial *matR*; and the nuclear 18S rDNA) for all 31 extant genera representing eight families of the order. At the familial level, the same phylogenetic relationships were inferred from five different analyses of these data. *Nothofagus*, followed by Fagaceae, were subsequent sisters to the rest of the order. Fagaceae are then sister to the core 'higher' hamamelids, which consist of two main subclades. The combined datasets provide the best-supported estimate of evolutionary relationships within Fagales. In addition, this study suggested that the combination of different sequences from several species within the same genus representing a terminal taxon had little influence on phylogenetic accuracy, and inclusion of taxa with some missing data in combined datasets did not have a major impact on the topology (Li *et al.* 2004).

The Lythraceae *sensu lato* (s.l.) comprise small to large trees, shrubs, and perennial and annual herbs adapted to a wide variety of vegetation types, including mangrove swamps, rainforests, seasonally dry savannahs, coastal dunes, freshwater marshes and shallow waters of ponds and rivers (Huang & Shi 2002). All members of the family share numerous specialized features with the rest of the order Myrtales. The phylogenetic relationships of Lythraceae s.l. were investigated by parsimony and likelihood analyses of 85 accessions representing 23 species; 16 genera have been assigned to the family at various times.

Phylogenetic analyses based on the three datasets (chloroplast *rbcL* gene, *psaA-ycf3* spacer and the nuclear ITS) strongly support the monophyly of the Lythraceae s.l., in which the satellite genera *Dubanga*, *Punica*, *Sonneratia* and *Trapa* were included. Paraphyly of subfamily Lythroideae (=Lythraceae *sensu stricto*) is proposed with the other four monotypic subfamilies nested within (Huang & Shi 2002).

There are many other examples of phylogenetic inferences at the intergeneric and higher levels and the application of molecular systematic approaches have resolved many debates involving a variety of vascular plant groups (Shi *et al.* 2000; Ge *et al.* 2002; Kong *et al.* 2002; Meng *et al.* 2003; Zhang *et al.* 2003a,b; Huang & Shi 2002; Guo & Ge 2005).

One of the important tasks of phylogenetic reconstruction is to reveal the closest relatives of model species and economically important crops. An obvious example is the study of the Asian cultivated rice (*Oryza sativa*) and its relatives. Rice is one of the world's most important crops and is becoming an excellent model for various biological studies, in particular after the completion of the rice genome projects (Yu *et al.* 2005). Although considerable effort has been devoted to studying rice genetics and breeding, the phylogeny of the rice genus (*Oryza*) has been less explored than that of the other major crops and model plants such as Arabidopsis, maize, wheat, soybean and cotton (Soltis & Soltis 2000). The recent decade has seen a series of molecular phylogenetic investigations undertaken in China to examine the relationships of rice and its relatives at different taxonomic levels. Based on the sequence data of two nuclear genes (*Adh1* and *Adh2*) and a chloroplast gene (*matK*), Ge *et al.* (1999) identified a new or the tenth genome type (HK) in the genus and reconstructed the phylogeny of all the 10 genomes and 23 *Oryza* species. The phylogeny indicated clearly that the G-genome species is the earliest divergent lineage, whereas the A-genome group, which contains the cultivated rice, is a recently diverged and rapidly radiated lineage within the rice genus. Further multiple-gene phylogenetic studies on the rice tribe based on chloroplast, mitochondrial and nuclear genes revealed that the rice tribe formed two monophyletic groups, and *Leersia* is the most closely related genus to *Oryza* (Ge *et al.* 2002; Guo & Ge 2005). To better understand the phylogenetic relationships of the Asian cultivated rice and its close relatives, which have been controversial for a long time, Zhu & Ge (2005) sequenced the fast evolving introns of four nuclear genes for eight A-genome species and reconstructed their phylogeny. The resulting phylogeny demonstrated that the Australian endemic, *O. meridionalis*, is the earliest divergent lineage and supported the previous opinion to treat *O. rufipogon* and *O. nivara* as a single species. More importantly, they indicated that two subspecies of *O. sativa* (ssp. *indica* and ssp. *japonica*) formed two separate monophyletic groups, suggestive of their independent origins from two ancestor lineages that separated from each other ca 400 000 years ago (Zhu & Ge 2005).

To put the divergence of the rice genus and tribe into a temporal framework, Guo & Ge (2005) and Zhu & Ge (2005) recently dated the divergence times of the

main lineages in the tribe and genus, respectively, based on the molecular clock approach. The estimates suggest that the rice tribe originated roughly 35 Myr ago, *Oryza* and *Leersia* separated from the rest of the tribe ca 20 Myr ago, and from each other 14 Myr ago. Within *Oryza*, the age of the deepest split between the most basal G genome and the remaining genomes was estimated at ca 9 Myr, and the diversification of the A-genome group occurred ca 2 Myr ago. Despite a number of limitations to the use of clocks based on sequence data, the divergence estimates for the main lineages in *Oryza* and its related genera provide a rough timeframe for understanding the evolutionary tempos of this important plant group and establish a framework for studying various biological questions using rice as a model system.

As the closely related group to rice, bamboos are a plant group of great ecological and economic importance in China. Based on partial sequences of the nuclear GBSSI gene and ribosomal ITS spacer, Guo & Li (2004) constructed the phylogeny of eight genera of the *Thamnocalamus* group and its allies that include the main food bamboos for the giant pandas and other rare fauna of the Himalayas and the adjacent areas. The results supported the monophyly of the *Thamnocalamus* group and its allies, but *Thamnocalamus* group *per se* was resolved as polyphyletic. In addition, the current limitation of *Thamnocalamus*, *Fargesia* (including *Borinda*) and *Yushania* may not reflect the true phylogenetic relationships of the complex. Kiwifruit (*Actinidia deliciosa*) is a popular fruit worldwide; it was domesticated from a single fruit collected in Central China and has been cultivated in New Zealand since 1904 (Li *et al.* 2002). China is both the distributional centre and the genetic diversity centre of *Actinidia* species, because 62 out of a total 66 species in this genus are found in China and high diversity exists within species. Based on molecular phylogenetic analyses of 23 *Actinidia* species, Li *et al.* (2002) found that neither ITS nor chloroplast *matK* phylogeny supported monophyly of any of the four sections of the genus, suggestive of the necessity of revision of infrageneric classification of *Actinidia*. In addition, polymorphic ITS sequences within accessions of some species implied historical events from hybridization or introgression. These, together with other examples, have highlighted the value of a phylogenetic framework in the effective utility and conservation management of the valuable genetic diversity.

5. MOLECULAR BIOGEOGRAPHY AND SPECIATION IN PLANTS

While China has the most diverse flora of any country in the North Temperate with very high levels of endemism, the diversity of plant genera and species is not uniformly distributed, but concentrated in the south central part of China (Ying 2001). Recent advance in phylogenetic reconstruction using DNA sequences provides an excellent opportunity for addressing biogeographic questions concerning plant biogeography and endemism.

The Qinghai–Tibet Plateau is the highest plateau in the world. Its short period of formation, since the

Pliocene, has considerably influenced the structure and evolution of its component flora (Shi *et al.* 1998). Although glaciations during the Quaternary period covered almost the entire plateau, and thus led to large-scale recession and extinction of many species, the plateau has an exceptionally diverse flora with approximately 4385 species of 1174 genera in 189 families. In contrast to the fact that over 25% of the total number of species are endemic to the plateau, less than 2% of the total number of genera have been considered endemic (Wu 1987), leading to a hypothesis that the Quaternary ice sheet had wiped out the flora of the plateau and the current flora migrated or originated from adjacent areas (Wulff 1944; Wu 1987).

Nannoglottis (Asteraceae) is a genus with about eight species endemic to the Qinghai–Tibet Plateau. Previous taxonomic studies have suggested its relationships with four different tribes of Asteraceae. Based on sequence data from chloroplast *ndhF* and *trnL-F*, and nuclear ITS, Liu *et al.* (2002) found that all sampled species of *Nannoglottis* formed a well-defined monophyly and were most closely related to the tribe Astereae. Despite the very early divergence of *Nannoglottis* in the Astereae, the tribe must be regarded to have its origin in the Southern Hemisphere rather than in Asia, because Asteraceae and its major lineages (tribes) are supposed to have originated in the Southern Hemisphere based on different lines of evidence (Liu *et al.* 2002). Since the 23–32 Myr divergence time between *Nannoglottis* and other Astereae predated the formation of the plateau, *Nannoglottis* seems to have reached the Qinghai–Tibet area in the Oligocene–Eocene and then re-diversified with the uplift of the plateau. Liu *et al.* (2002) also estimated that the ‘alpine shrub’ versus ‘coniferous forest’ divergence within *Nannoglottis* was *ca* 3.4 Myr ago, when the plateau began its first large-scale uplifting and the coniferous vegetation began to appear.

Most of the current species at the ‘coniferous forest’ clade of the genus are estimated to have originated from 1.02 to 1.94 Myr ago, when the second and third uprisings of the plateau occurred. The diversification and radiation of *Nannoglottis* in the Qinghai–Tibet Plateau suggested by the molecular phylogenetic study agreed well with the known geological and palaeobotanical histories of the Qinghai–Tibet Plateau (Liu *et al.* 2002). Similarly, Yang *et al.* (2003) also detected a high level of ITS divergence for 42 *Pedicularis* species sampled from this region and suggested a relatively ancient origin and diversification of the genus followed by migration of different floristic components into this region.

The Hengduan Mountain region is located in the southeast part of the Qinghai–Tibet Plateau and is one of the three Chinese hotspots of plant diversity (Ying 2001). The uplift of the Qinghai–Tibet Plateau in the past 40 Myr has caused the regional climate to cool down markedly and changed the regional topography. This has led to a sharp change in the vegetation and made the area an important centre of survival, speciation and evolution (Ying 2001). An excellent example is *Pinus densata*, which is distributed in the Hengduan Mountain region and the adjacent area.

Previous morphological, allozyme and chloroplast DNA data indicated that this species is a hybrid pine originated from hybridization between *P. tabulaeformis* and *P. yunnanensis* and the different populations of *P. densata* have different evolutionary histories (Wang & Szmidi 1994). Further analyses of allozyme and restriction sites of chloroplast and mitochondrial fragments based on population samples revealed different evolutionary histories among *P. densata* populations and that *P. tabulaeformis* and *P. yunnanensis* had acted as both mother and father donors (Wang *et al.* 2001; Song *et al.* 2003). In addition, *P. densata* populations have a stabilized hybrid nature and maintained successful sexual reproduction with normal fertility and high fecundity despite significant founder effects; the backcross happened during the population establishment of the hybrid pine (Wang *et al.* 2001; Song *et al.* 2003). *P. densata* represents an example of diploid hybrid speciation in an extreme ecological habitat that is both spatially and ecologically separated from that of its parents.

Polyploidization followed by hybridization between species is another force driving plant speciation. Low-copy nuclear genes, which contain fast-evolving introns and are less susceptible to concerted evolution, are powerful markers for reconstructing allopolyploidization. Using sequences of biparental inherited nuclear *Adh1* and *Adh2* genes as well as the maternally inherited *matK* gene, Ge *et al.* (1999) reconstructed the origin of the allotetraploid species in *Oryza*. In contrast to a single origin of three CD-genome species, the BC-genome species had different origins because their maternal parents had either a B or a C genome (Ge *et al.* 1999). A study with additional genes and samples further demonstrated that the CD-genome species originated from the hybridization between the C- and E-genome species, and that the C-genome species served as the maternal parent (Bao & Ge 2004). Zhang *et al.* (2002a–c) also studied the origin and evolution of some tetraploid wheat using nuclear ITS sequences.

6. POPULATION AND CONSERVATION GENETICS OF ANIMALS

Knowledge of population history and genetic structure is not only necessary to understand the evolutionary process of the species, but also useful for conserving the endangered species.

Liu *et al.* (2006a) studied the demographic history and genetic structure of *Lateolabrax maculatus* and *L. japonicus* in the northwestern Pacific using the cytochrome *b* gene and control region sequences. The demographic history of the two species was examined using neutrality tests and mismatch distribution analyses, and results indicated Pleistocene population expansion in both the species. Estimates of population expansion time suggested earlier population expansion in *L. japonicus* than in *L. maculatus*. Molecular variance analyses showed differential genetic structuring for these two closely related species. Their results indicated that *L. japonicus* is panmictic throughout its range. In contrast, populations of *L. maculatus* showed statistically significant levels of genetic structuring. Pattern of isolation by distance was observed in

L. maculatus, suggesting that *L. maculatus* is in genetic equilibrium. In contrast, *L. japonicus* did not exhibit isolation by distance (Liu *et al.* 2006a). Such kind of study can provide rich information on the population history and the genetic structure of animal species.

Climatic oscillations during the Pleistocene ice ages produced great changes in species' geographical distribution and abundance, which could be expected to have genetic consequences. To investigate the effects of Pleistocene climatic changes on the evolution in Japanese anchovy, Liu *et al.* (2006b) determined mtDNA control region sequences for 241 individuals from 13 localities and 37 individuals of Australian anchovy. Japanese anchovy and Australian anchovy are reciprocally monophyletic and a Late Pleistocene transequatorial divergence between the two species was indicated. Analyses of molecular variance and the conventional population statistic F_{ST} revealed no significant genetic structure throughout the range of Japanese anchovy. Both mismatch distribution analyses and neutrality tests suggested a Late Pleistocene population expansion for both Japanese anchovy (79 000–317 000 years ago) and Australian anchovy (45 000–178 000 years ago; Liu *et al.* 2006b).

Combining microsatellites with mitochondrial sequences is useful for studying the genetic structure of animal species. Shao *et al.* (2004) investigated seven mainland and island Asian populations of *Bombus ignitus* using nine microsatellite markers and the mitochondrial cytochrome *b* (*cytb*) gene sequences. While microsatellite markers showed high genetic variability, no sequence variation was found in the *cytb* gene fragment analysed. Gene diversities per locus per population ranged from 0.378 to 0.992. AMOVA test and most pairwise F_{ST} values showed significantly genetic differentiation between mainland and island populations. The *cytb* sequence data and microsatellite bottleneck tests indicated that almost all populations were subjected to recent bottleneck. Their results suggest that *B. ignitus* populations diverged due to recent bottleneck and geographical isolation (Shao *et al.* 2004).

It is generally believed that there is a negative correlation between the level of genetic diversity and the endangerment of a species. However, what much genetic diversity really reflects is the demographic history of the species rather than its fitness.

A recent study of Li *et al.* (2003) provided an example of a proper interpretation of the genetic diversity. The Golden monkey (*Rhinopithecus roxellana*) is a well-known primate, which is distributed in the central part of mainland China, with a population size around 10 000–20 000. Forty-four allozyme loci were surveyed in 32 individuals, none of which were found to be polymorphic. The void of polymorphism compared with that of other non-human primates is surprising, particularly considering that the current population size is many times larger than some other endangered species. Coalescent approaches were used to explore various scenarios of population bottleneck, and it was concluded that the most recent bottleneck could have happened within the last 15 000 years. Moreover, the proposed simulation approach could be useful for researchers who need to analyse zero or low polymorphism data (Li *et al.* 2003).

The red panda (*Ailurus fulgens*) is an endangered species and its present distribution is restricted to the isolated mountain ranges in western China (Sichuan, Yunnan, and Tibet provinces) and the Himalayan Mountain chain of Nepal, India, Bhutan and Burma. Li *et al.* (2005a) did not observe significant differentiation between subspecies. They suggest that the present population structure has resulted from habitat fragmentation and expansion from glacial refugees. Owing to its habitat requirements, it is probable that the red panda has undergone bottlenecks and population expansions several times in the recent past. The present population may exhibit a pattern reminiscent of a relatively recent population expansion. Similar to the red panda, significant genetic differentiation has not been detected among the giant panda populations, and a population bottleneck was inferred (Zhang *et al.* 2002a–c).

The Chinese sturgeon, *Acipenser sinensis*, is an endangered anadromous fish, mainly endemic to the Yangtze River, China. Its spawning migratory route was blocked in 1981 by the Gezhouba Dam, which caused a drastic decline of the natural population. Zhang *et al.* (2003a,b) investigated mtDNA sequence variation in this species. Their results indicate that the Chinese sturgeon underwent a population expansion in the past, but there is no indication of a historic genetic bottleneck. Strikingly, the ratios of effective female population size (N_{ef}) to the census female population size (N_f) are unusually high (0.77–0.93). This may be interpreted as the result of a current or recent bottleneck in the population, which is more probably caused by human intervention, rather than evolutionary forces (Zhang *et al.* 2003a,b).

These studies demonstrate how population genetic analysis can help to understand an endangered species. A species with low genetic diversity could have the ability to expand if the habitat is conserved.

7. ORIGIN OF DOMESTIC ANIMALS

The domestic animals were extremely important in the process of human civilization. Understanding the origin and dispersal of domestic animals is not only important by itself, but can also provide valuable information to trace the recent human evolution as well.

Archaeological finds from Mesolithic sites around the world indicate that the dog was the first domestic animal. The origin of the domestic dog from wolves was established, but the number of founding events, as well as where and when these occurred, is not known. To address these questions, Savolainen *et al.* (2002) examined the mtDNA sequence variation among domestic dogs representing all major populations worldwide. They found that more than 95% of all the sequences belonged to three universally represented phylogenetic groups, showing a common origin from a single gene pool for all dog populations. A larger genetic variation in East Asia compared with Southwest Asia and Europe strongly suggests an East Asian origin *ca* 15 000 years ago for the domestic dog (Savolainen *et al.* 2002).

Domestic chickens have long been important to human societies for food, religious, entertainment and decorative uses, yet the origin and diffusion of chickens

in the world remain unclear. Liu *et al.* (2006c) assessed the origin of chicken by analysing the mtDNA sequences from 834 domestic chickens across Eurasia as well as 66 red jungle fowls (*Gallus gallus*) from Southeast Asia and China. They revealed nine highly divergent mtDNA clades (A–I), of which seven clades contained both the red jungle fowls and domestic chickens. The clades A, B and E are distributed ubiquitously in Eurasia, while the other clades were restricted to South and Southeast Asia. Clade C was mainly distributed in Japan and Southeast China, while clades F and G were exclusive to Yunnan, China. The geographical distribution of clade D was closely related to the distribution of the pastime of cock fighting. Statistical tests detect population expansion within each subclade. These distinct distribution patterns and expansion signatures suggest that different clades may originate from different regions, such as Yunnan, South and Southwest China and/or surrounding areas, and the Indian subcontinent, respectively, which support the theory of multiple origins in South and Southeast Asia (Liu *et al.* 2005).

China has numerous native domestic goat breeds, but so far there has been no extensive study on genetic diversity, population demographic history and origin of Chinese goats. Chen *et al.* (2005) examined the genetic diversity and phylogeographic structure of Chinese domestic goats. They revealed that there were four mtDNA lineages (A, B, C and D) identified in Chinese goats, in which lineage A was predominant, lineage B was moderate and lineages C and D were at low frequency. These results further support the multiple maternal origins of domestic goats. They detected two subclades in lineage B, in which one was unique to eastern Asia, another was shared between eastern and southern Asia. A larger genetic variation in eastern Asia than southern Asia and the pattern of phylogeographic variation in lineage B suggest that at least one subclade of lineage B originated from eastern Asia. There was no significantly geographical structuring in Chinese goat populations, which suggested that there existed strong gene flow among goat populations caused by extensive transportation of goats in history (Chen *et al.* 2005).

To investigate the origin and genetic diversity of Chinese domestic sheep, Chen *et al.* (2006) analysed mtDNA control region of 449 Chinese autochthonous sheep from 19 breeds/populations from 13 geographical regions. Phylogenetic analysis showed that all the three previously defined lineages A, B and C were found in all sampled Chinese sheep populations, except for the absence of lineage C in four populations. The pattern of genetic variation in lineage A, together with the divergence time between the two central founder haplotypes, suggested that two independent domestication events have occurred in sheep lineage A. The high levels of intrapopulation diversity in Chinese sheep and the weak phylogeographic structuring indicated that three geographically independent domestication events have occurred and the domestication place was not only confined to the Near East, but also occurred in other regions (Chen *et al.* 2006).

These and other studies suggest that East and South Asia is one of the centres for domestication, which provides a good opportunity for Chinese scientists.

Further studies using more genetic markers, and on other domestic animals in this region, will shed new light on the origin of domestic animals.

8. ORIGIN OF GENES

Origin of genes is a fundamental process during the evolution of organisms (Long *et al.* 2003). The mechanism of origin of genes and the evolutionary forces behind the functional divergence of newly originated genes are not yet fully understood. Genome-wide analysis and phylogenetic inference of genes and gene families have shed some light on this issue.

Recently, Wang *et al.* (2004) discovered a unique young gene family of *Drosophila*. The new gene family was named *monkey-king* (*mkg*) following an ancient Chinese legend in which the monkey king could instantly produce many offspring transformed from its hairs, to account for several new genes that were created in this gene family in a very short time. This *mkg* family could be used to answer a number of important questions in the study of new gene origination. First, they directly showed how a eukaryotic gene fissions for the first time. The process by which *mkg* originated demonstrates that gene duplication followed by complementary partial degeneration is a simple, but efficient mechanism for gene fission. This also provides a general mechanism to generate new introns in a previously intronless gene. In addition, this gene family also showed that regulatory sequences could be created quickly (Wang *et al.* 2004).

Complete genome sequences demonstrated that duplication is a major source for the origin of genes. It is widely accepted that there is often an acceleration of the rate of evolution following gene duplication, which could be driven either by relaxation of selective constraints or by positive selection. However, the relative contribution of these two factors is still controversial. A natural question is the role of gene duplication in organismal adaptation and biodiversity (Zhang *et al.* 2002a–c).

Growth hormone (GH) is a classic molecule in the study of the molecular clock hypothesis, as it exhibits a relatively constant rate of evolution in most mammalian orders except primates and artiodactyls, where dramatically enhanced rates of evolution (25- to 50-fold) have been reported. Ye *et al.* (2005) determined 21 different GH-like sequences from four species of OWM and Hominoids. Their analysis demonstrated that multiple gene duplications and several gene conversion events both occurred in the evolutionary history of this gene family in OWM/Hominoids.

GH-N genes in hominoids and OWM are under strong purifying selection. In contrast, *CSH* genes in both lineages are probably not. *GH-V* genes in OWM and hominoids evolved at different evolutionary rates and underwent different selective constraints. Recent phylogenetic analysis of Li *et al.* (2005c) revealed monophyly for New World monkey (NWM) *GH*-like genes with respect to those of OWM/hominoids, which indicates that independent gene duplications have occurred in NWM *GH*-like genes. Their results disclosed the complex history of the primate *GH* gene

family and raised intriguing questions on the consequences of these evolutionary events.

Wang *et al.* (2005a,b) identified and characterized three *Spindlin* (*Spin*) genes from medaka (*Oryzias latipes*). Their phylogenetic analysis demonstrated that those three genes resulted from gene duplication. Furthermore, Western blot analysis revealed significant expression differences of the three *OlSpins* among different tissues and during embryogenesis in medaka, and suggested that sequence and functional divergence might have occurred during evolution.

The enzyme chalcone synthase (CHS) is a key enzyme in the biosynthesis of flavonoids, which are important for the pigmentation of flowers and act as attractants to the pollinators. The *CHS* gene family is probably one of the most studied gene families in plants. Growing evidence shows that genes encoding CHS constitute a multigene family in which the copy number varies among plant species and functional divergence appears to have occurred repeatedly (Yang *et al.* 2002). Plants in the genus *Dendranthema* (Asteraceae) have white, yellow and pink flowers, exhibiting considerable variation in flower colour. Based on the phylogenetic analyses of the 18 *CHS* genes cloned and sequenced from six *Dendranthema* species and those available for other genera of Asteraceae in GenBank, Yang *et al.* (2002) found that except for two pseudogenes, the functional *Dendranthema CHS* genes formed three well-supported subfamilies: SF1, SF2 and SF3. The inferred phylogeny of the *CHS* genes of *Dendranthema* and *Gerbera* suggests that these genes originated as a result of duplications before divergence of these two genera, and the function of *Dendranthema CHS* genes have diverged in a similar fashion to the *Gerbera CHS* genes, i.e. the genes of SF1 and SF3 code for typical CHS enzymes expressed during different stages of development, whereas the genes of SF2 code for another enzyme that is different from CHS in substrate specificity and reaction. More importantly, much higher non-synonymous–synonymous rate ratio for the lineage ancestral to SF2 was detected, suggesting that positive selection appeared to have driven the divergence of SF2 from SF1 and SF3 (Yang *et al.* 2002).

Gene duplication may arise from region-specific duplication or genome-wide polyploidization, and ranks among the most important evolutionary mechanisms affecting plant genome evolution. Since gymnosperms are characterized by a large nuclear genome and highly complex gene families, it is of interest to investigate the frequency of gene duplication and the evolutionary fate and the consequences of duplicate genes. Wang *et al.* (2000) studied intergeneric relationships of Pinaceae using a low-copy nuclear *4CL* gene and found that duplication and deletion of the *4CL* gene occurred at a tempo such that paralogous loci were maintained within but not between genera. They also found that exons of the *4CL* gene have diverged approximately twice as fast as the *matK* gene and five times more rapidly than the *nad5* gene. Further investigation on the evolution of *4CL* gene in *Larix*, another genus in Pinaceae, demonstrated that frequent duplication/deletion appears to be a common evolutionary phenomenon. This gene family and

paralogous genes differ greatly in their evolution rate (Wei & Wang 2004).

To examine whether the amino acid substitution rates are the same between duplicate genes of a ubiquitous NF-Y transcription factor, Yang *et al.* (2005a,b) used likelihood-ratio tests to evaluate the influence of selection on evolution of duplicated *NF-Y* genes in the *Arabidopsis* and rice genomes by comparing the conservative and radical amino acid substitution rates. The results indicated that some NF-YB and NF-YC duplicates showed significant evidence of asymmetric evolution, but not the NF-YA duplicates, and most amino acid replacements in the NF-YB and NF-YC duplicates resulted in changes in hydrophobicity, polar requirement and polarity. They suggested that relaxed selective constraints following gene duplication are most probably responsible for the unequal evolutionary rates and distinct divergence patterns of duplicate *NF-Y* genes, and positive selection might have promoted amino acid hydrophobicity changes in the NF-YC duplicates (Yang *et al.* 2005a,b).

These studies support the idea that relaxation of selective constraints is common following gene duplication, but positive selection may operate to drive the function divergence among duplicates.

9. EVOLUTION OF GENES

SRY is a Y-chromosomal gene that is pivotal in initiating the development of testis and a determinant of male sex in mammals. A higher rate of non-synonymous nucleotide substitution than that of synonymous substitution in the terminal regions of *SRY* has been reported in primates, with suggestions that these regions may be subject to positive Darwinian selection (Whitfield *et al.* 1993). Wang *et al.* (2002) sequenced the *SRY* genes of nine OWMs and revealed reduced rate of non-synonymous substitution and action of purifying selection in the terminal regions of *SRY* in OWMs. While confirming earlier results of high non-synonymous substitution rates of *SRY* in hominoids (Whitfield *et al.* 1993), they were not able to reject the neutral evolution hypothesis. As such, whether the rapid evolution of *SRY* in hominoids is due to positive selection or relaxed functional constraints remains an open question. Thus, *SRY* shows a complex pattern of erratic evolution among different groups of primates, raising an intriguing possibility of varied selective pressure on this fundamentally important gene in evolution (Wang *et al.* 2002).

Yang & Gui (2004) studied transferrin polymorphism in the three subspecies of polyploid *Carassius auratus*. DNA polymorphism of extremely high extent was shown for the transferrin gene by the 248 segregation sites among coding region sequences of its alleles. The deduced amino acid sequences of the transferrin alleles showed variable theoretical physicochemical parameters; positive selection was observed. Furthermore, the corresponding sites to these selected codons were collectively located at two planes in the crystallographic structure of rabbit transferrin, which suggested that the rapid evolution of *C. auratus* transferrin might correlate to its adaptation to variable environmental elements such as oxygen pressure. The minimal 26 recombination

events were detected among coding sequences of *C. auratus* transferrin, with partial mosaic sequences and breakpoints. Phylogenetic analyses revealed multiple antique allelic lineages of transferrin, which was estimated to diverge 15–20 Myr ago. All these features strongly suggested the role of balancing selection in long persistence of high transferrin polymorphism in *C. auratus* (Yang & Gui 2004).

Protein tyrosine phosphatase (PTP) consists of two superfamilies, the phosphatase I with a single low-molecular-weight PTP (lmwPTP) family and the phosphatase II including both the higher-molecular-weight PTP (hmwPTP) and the dual specificity phosphatase (DSP) families, but the phosphatases I and II are usually considered to be the result of convergent evolution (Stone & Dixon 1994; Barford *et al.* 1995). However, Huang (2003) demonstrated that lmwPTPs and hmwPTPs/DSPs are remotely related in evolution, and all these three PTP families might have resulted from a common ancestral gene by a series of duplications, fusions and circular permutations. The circular permutation in PTPs is caused by a reading frame difference, which is similar to that in DNA methyltransferases (Jeltsch 1999). Nevertheless, the evolutionary mechanism of circular permutation in PTP genes seems to be more complicated than that in DNA methyltransferase genes. Both mechanisms in PTPs and DNA methyltransferases can be used to explain how some protein families and superfamilies came to be formed by circular permutations during molecular evolution.

Glycyl-tRNA synthetases (GlyRSs) with $\alpha 2\beta 2$ tetramer are distributed in most bacteria, but those with $\alpha 2$ dimer are in archaeobacteria, eukaryotes and a few bacteria. Tan & Huang (2005) found that the anticodon-binding domains (ABDs) of dimeric and tetrameric GlyRSs are non-homologous, but their catalytic central domains are homologous. The ABD structures of dimeric and tetrameric GlyRSs are clearly different, but the functions of both GlyRSs in dimer and tetramer forms are the same. Thus, the mechanisms of GlyRS recognizing tRNA^{Gly} in dimeric and tetrameric GlyRSs should be different. The results suggest that the same function in some proteins can be realized by mechanisms changing during evolution from bacteria to eukaryotes, which also implies a special evolutionary way in GlyRS recognizing tRNA^{Gly}.

Divergence of proteins in signalling pathways requires ligand and receptor coevolution to maintain or improve binding affinity and/or specificity. Li *et al.* (2005b) provided a clear case of coevolution between the prolactin (PRL) gene and its receptor (prolactin receptor, PRLR) in mammals. First, they observed episodic evolution of the extracellular domain (ECD) and the intracellular domain (ICD) of the PRLR, which is closely consistent with that seen in PRL. Correlated evolution was demonstrated both between PRL and its receptor and between the two domains of the PRLR using Pearson's correlation coefficient. On comparing the ratio of the non-synonymous substitution rate to synonymous substitution rate (ω) for each branch of the star phylogeny of mammalian PRLR, separately for the ECD and the transmembrane domain/intracellular domain (TMD/ICD), they

observed a lower ω ratio for ECD than TMD/ICD along those branches leading to pig, dog and rabbit, but a higher ratio for ECD than TMD/ICD on the branches leading to primates, rodents and ruminants, on which bursts of rapid evolution were observed. These observations can be best explained by coevolution between PRL and its receptor and between the two domains of the PRLR (Li *et al.* 2005b).

To better understand the evolution of genes, it is necessary to take network interaction of genes into account in future studies.

10. FROM SINGLE GENE TO GENOME APPROACH

In the genomic era, it is evident that there is a transition from single-gene investigation to a genomic approach.

The explosion of genomic studies in *Arabidopsis*, rice, maize and other model plants is providing unprecedented opportunities to address many biological questions, including evolution of gene and genome. For example, members of the *GRAS* gene family encode transcriptional regulators that have diverse functions in plant growth and development such as gibberellin signal transduction, root radial patterning, axillary meristem formation, phytochrome A signal transduction and gametogenesis (Tian *et al.* 2004). To take full use of the *Arabidopsis* and rice genome sequences and bioinformatic approaches, Tian *et al.* (2004) identified 57 and 32 *GRAS* genes in rice and *Arabidopsis*, respectively, and the *GRAS* gene family could be divided into eight subfamilies, with distinct conserved domains and functions. They also found that both genomic/segmental duplication and tandem duplication contributed to the expansion of the *GRAS* gene family in the rice and *Arabidopsis* genomes. The existence of *GRAS*-like genes in bryophytes suggests that *GRAS* is an ancient family of transcription factors, which arose before the appearance of land plants over 400 Myr ago (Tian *et al.* 2004).

S-phase kinase-associated protein 1 (SKP1) is a core component of SCF ubiquitin ligases and mediates protein degradation, thereby regulating eukaryotic fundamental processes. Despite a single *SKP1* gene in protists, fungi and some vertebrates, many animal and plant species possess multiple *SKP1* homologues. By comparing the entire sets of *SKP1* homologues of rice and *Arabidopsis* as well as fungi and animals, Kong *et al.* (2004) demonstrated that multiple *SKP1* homologues from the same species have evolved at highly heterogeneous rates and that the differences in evolutionary rate were so large that true phylogenies were not recoverable from the full dataset. They indicated that only when the original dataset was partitioned into sets of genes with slow, medium and rapid rates of evolution and analysed separately, better-resolved relationships were observed. They divided the *SKP1* genes into three categories. The first are the slowly evolving *SKP1* homologues that are relatively highly conserved in sequence and expressed widely and/or at high levels, suggesting that they have evolved under functional constraint and served the most fundamental function(s). The second category consists of the rapidly evolving members that are structurally more diverse and usually have limited expression patterns and higher

dN/dS values, suggesting that they might have evolved under relaxed or altered constraint, or even under positive selection. The final category includes some rapidly evolving members that might have lost their original function(s) and/or acquired new function(s) or become pseudogenes, as suggested by their expression patterns, dN/dS values, and amino acid changes at key positions (Kong *et al.* 2004).

In the rice genome, large-scale duplication events have been recently uncovered, but different interpretations (aneuploid or palaeopolyploid) were proposed regarding the extent of the duplications based on the draft sequence of the genome of the subspecies *japonica* (Paterson *et al.* 2005). Using independent dating approaches and the genome sequence data of the other subspecies of rice (*indica*), Wang *et al.* (2005a,b) detected 10 duplicated blocks on all 12 chromosomes and thus supported whole-genome duplication. In addition, they inferred that the whole-genome duplication occurred *ca* 70 Myr ago and a segmental duplication at *ca* 5 Myr ago, involving chromosomes 11 and 12. Yu *et al.* (2005) published an improved, near-complete genome analysis of two subspecies of rice and also reported that there was evidence in the rice DNA sequences for a whole-genome duplication *ca* 55–70 Myr ago.

Shi *et al.* (2003) identified 10 and 30 putative bitter taste receptor *T2R* genes from the draft human and mouse genome sequences, respectively. Their phylogenetic analysis of the *T2R* genes suggests that these genes can be classified into three main groups, i.e. A, B and C. They demonstrated that tandem gene duplication is the primary source of new *T2Rs*. For closely related paralogous genes, a significantly higher rate of non-synonymous nucleotide substitution than that of synonymous substitution was observed in the extracellular regions of *T2Rs*, which are presumably involved in tastant-binding. This suggests the role of positive selection in the diversification of newly duplicated *T2R* genes. Since many natural poisonous substances are bitter, they conjectured that the mammalian *T2R* genes are under diversifying selection for the ability to recognize a diverse array of poisons that the organisms may encounter in exploring new habitats and diets.

In mammals, the vomeronasal organ (VNO) of the olfactory system is a chemosensory organ specialized in the detection of pheromones, chemical signals that induce innate reproductive and social behaviours between the members of the same species. The pheromone receptors are encoded by two distinct and complex superfamilies, named *V1R* and *V2R*.

Shi *et al.* (2005) used computational methods to identify 95 and 62 new putative *V1R* genes from the draft rat and mouse genome sequence, respectively. The rat *V1R* repertoire consists of 11 subfamilies, 10 of which are shared with the mouse while the rat appears to lack the H and I subfamilies found in mouse and possesses one unique subfamily (M). Their analysis reveals that many subfamilies underwent an expansion very close to the split of mouse and rat. The non-synonymous and synonymous rate ratios for most of these clusters were much higher than one, suggesting the role of positive selection in the diversification of

these duplicated *V1R* genes. They speculate that the *V1R* genes have evolved under positive Darwinian selection to maintain the ability to discriminate between large and complex pheromonal mixtures. Grus *et al.* (2005) describe the *V1R* repertoires of dog, cow and opossum, based on their draft genome sequences. Phylogenetic analysis of placental *V1R* genes suggests multiple losses of ancestral genes in carnivores and artiodactyls and gains of many new genes by gene duplication in rodents, manifesting massive gene births and deaths. Their results show a concordance between the *V1R* repertoire size and the complexity of VNO morphology, suggesting that the latter could indicate the sophistication of pheromone communications within species (Grus *et al.* 2005).

Based on a computational analysis of the mouse and rat genome sequences, Yang *et al.* (2005a,b) report the first global draft of the *V2R* vomeronasal receptor gene repertoire, composed of approximately 200 genes and pseudogenes. Rodent *V2Rs* are subject to rapid gene births/deaths and accelerated amino acid substitutions, probably reflecting the species-specific nature of pheromones. Vertebrate *V2Rs* appear to have originated twice prior to the emergence of the VNO in ancestral tetrapods.

Those studies demonstrate that the genomic approach is very powerful and necessary for understanding the evolution of genes and gene families.

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