

Comparative phylogeography of four *Apodemus* species (Mammalia: Rodentia) in the Asian Far East: evidence of Quaternary climatic changes in their genetic structure

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The phylogeography of four *Apodemus* species (*Apodemus agrarius*, *Apodemus peninsulae*, *Apodemus latronum*, and *Apodemus draco*) was studied in the Far East of Asia, based on sequences of the mitochondrial DNA cytochrome *b* gene. The results obtained show the existence of many different genetic lineages within the studied *Apodemus* species, suggesting the isolation and differentiation of populations in multiple refuge areas. Higher genetic diversities in some regions such as Yunnan, Sichuan (China), and eastern Russia suggest these areas are potential refuges for these species. The existence of such complex genetic structures could be linked to the presence of many biogeographic barriers (Himalaya Mountains, Tien-shan Mountains, Altai Mountains, Tibetan Plateau, Gobi desert, Yunnan Guizhou Plateau, Dzungaria basin, and others) in these regions, which were probably reinforced during the Quaternary climate changes. These barriers also played an important role concerning the low dispersal abilities of the two studied *Apodemus* species adapted to forest habitats (*A. latronum* and *A. draco*) with respect to colonizing regions other than China. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 797–821.

ADDITIONAL KEYWORDS: China – Russia – glacial refuges.

INTRODUCTION

In recent years, a great number of phylogeographic studies have been published on European plants, insects, amphibians, birds, fishes, and several

mammals (Taberlet *et al.*, 1998; Avise, 2000; Hewitt, 2001). They have shown the importance of Pleistocene climate changes and biogeographic barriers such as mountains, rivers, seas, and deserts for the diversification, radiation, and isolation of new genetic lineages within many species (Avise, 2000; Riddle *et al.*, 2000). These climatic oscillations and particular topographies played a major role in shaping of the

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present geographical distribution of species, including their genetic structure and diversity (Avice, 1994). This phenomenon has resulted in the extinction of the northern populations of many species during ice ages, followed by subsequent northward expansions from refugia during interglacial periods (Hewitt, 1996, 1999, 2000; Taberlet *et al.*, 1998). Avice (1994) and Hewitt (1996) postulated that the rapid expansion from refugial populations involved periodic bottlenecking with a progressive loss of allelic diversity. This would result in lower genetic diversity in populations present in the more recently colonized places. By contrast, it would be expected that the populations remaining in the refugia would be less affected by climatic changes and would be more genetically diverse. The isolation of populations of many species in separate southern regions during ice ages could have resulted in an allopatric differentiation into several genetic groups that recolonized the western Palaearctic region in different ways at the end of the last ice age.

Many phylogeographic studies were also carried out in other regions such as North America (Funk *et al.*, 2008; Hull *et al.*, 2008; Aubry *et al.*, 2009; Latch *et al.*, 2009; Timpe, Graham & Bonett, 2009), Central and South America (Da Silva & Patton, 1998; Paris *et al.*, 2000; Cabanne *et al.*, 2008; Zemlak *et al.*, 2008), as well as in Australia (Waters & Roy, 2003; Miller & Allsopp, 2005; Carini & Hughes, 2006). Similar to Europe, they demonstrated complex phylogeographic patterns for these regions. These patterns also appear to be linked to the extension of the ice sheets during the Pleistocene climate changes, the fragmentation of forests, and the aridification of some regions, as well as to the presence of biogeographic barriers such as the Rockies, the Appalachian, and the Andean mountains (Hewitt, 2000).

Concerning regions in Asian Far East, the glacial advances were not as extensive as in Europe or in America because of the influence of the monsoons from south-east Asia and the increasingly arid climates resulting from the Quaternary uplift of the Tibetan plateau (Zhou *et al.*, 2004). However, geological data have demonstrated that, during the Last Glacial Maximum, ice sheets five- to seven-fold larger than the current ones covered the Tibetan Plateau. Moreover, paleoecological biome reconstructions and palynological data (Harrison, Yu & Takahara, 2001) have indicated that, during the same period, the steppe and desert vegetation extended to the modern coastline of eastern China instead of the temperate deciduous forests currently found over the same area. The boundary of coniferous forests probably shifted 300–1000 km south of 30°N during this period (Chen *et al.*, 2008).

Although coniferous and deciduous forests most likely recolonized a part of northern China during the

interglacial periods (Chen *et al.*, 2008), the climate recoveries were unlike those of other regions owing to the continuing uplift of the Tibetan Plateau (Zhou *et al.*, 2004). Indeed, this geological phenomenon, considered to be one of the most important events of the Quaternary period, caused great climatic changes even during the interglacial periods, with grasslands replacing forests in many regions as the climate gradually became drier, colder, and windier (Wu *et al.*, 2001).

Despite the relatively 'milder' climate than the one in other regions in the world, the frequent arid phases and changes of vegetation of many Chinese regions during the Quaternary climatic fluctuations probably strongly influenced the evolution and distribution of many species (Duan *et al.*, 2009).

However, excepting partial information on the red deer (*Cervus elaphus*) (Mahmut *et al.*, 2002; Ludt *et al.*, 2004), the lesser white-toothed shrew (*Crocidura suaveolens*) (Dubey *et al.*, 2006), the red panda (*Ailurus fulgens*) (Li *et al.*, 2005), the harvest mouse (*Micromys minutus*) (Yasuda *et al.*, 2005), the greater long-tailed hamster (*Tscherskia triton*) (Xie & Zhang, 2005) or the Caspian tiger (*Panthera tigris virgata*) (Driscoll *et al.*, 2009), very few phylogeographic studies have been carried out on mammal species from these regions.

To better understand the phylogeographic structure of mammal species from Asian Far East or the east Palaearctic, we chose to study the phylogeographic structure of four *Apodemus* Kaup, 1829 species (Rodentia, Muridae) from this region: *Apodemus agrarius* Pallas, 1771, *Apodemus peninsulae* Thomas, 1906, *Apodemus latronum* Thomas, 1911, and *Apodemus draco* Barrett-Hamilton, 1900 (Smith & Xie, 2008).

Species of the genus *Apodemus* are the most common rodents in broad-leaf forests in the temperate zone of the Palaearctic region (Corbet, 1978; Corbet & Hill, 1992). They generally depend on forest resources, such as seeds and small invertebrates. *Apodemus latronum* and *A. draco* are particularly associated with forests (Vaniscotte *et al.*, 2009). By contrast, *A. agrarius* and *A. peninsulae* are commonly observed in more open habitats such as shrubs, field banks, slope grass, sparse bushes or steppes (Smith & Xie, 2008; Vaniscotte *et al.*, 2009).

Apodemus species have limited species-specific distributions, with two or more species often cohabiting the same forest (Corbet, 1978). Recent phylogenetic analyses (Michaux *et al.*, 2002; Suzuki *et al.*, 2008) demonstrated that these East Asian *Apodemus* species appeared after a two-step radiation process: the first step occurring approximately 4.5–5 Mya, gave rise to *A. agrarius* and the ancestor of *A. draco*/*A. latronum* and *A. peninsulae*. The second

step took place approximately 2 Mya when *A. draco* and *A. latronum* diverged, probably after an allopatric or parapatric speciation (Suzuki *et al.*, 2008). These radiation events were most likely primed by global changes that occurred 5–6 Mya and 2–3 Mya (beginning of the Quaternary period) (Michaux *et al.*, 2002). Therefore, because these species have been present and diversified in this area for several millions of years, they represent interesting models by which to better understand the impact of past climate fluctuations on their genetic diversity.

More precisely, the present study aimed to answer several questions: Do these *Apodemus* species display intraspecific genetic diversity? If so, is this genetic diversity geographically structured? Could it be associated with the very complex topography characterizing these regions, particularly in China (i.e. the Himalaya Mountains, the deep river valleys between recently uplifted mountains or the Gobi desert)? As in other regions of the world, did the Quaternary climate fluctuations play a role in this intraspecific genetic differentiation and, if so, at what level? What was demographic history of the species during this period?

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

We analyzed the tissues of a total of 118 specimens of four *Apodemus* species (20 *A. draco*, 19 *A. latronum*, 25 *A. peninsulae*, 54 *A. agrarius*) from China and the Russian Far East localities (Table 1) and widespread throughout their Asian Far East distribution area (Fig. 1A, B, C, D). These tissues came from the *Apodemus* tissue collection of the Center of Biology and Management of Populations of Montpellier (CBGP), France (curator J.P. Quéré) (Giraudoux *et al.*, 2008; Raoul *et al.*, 2008), the collection of Drs M. Pavlenko, I. Kartavtseva, and G. Chelomina (housed at the Institute of Biology and Soil Science, Far East Branch of Russian Academy Science, Vladivostok, Russia), as well as the collection of Dr A. Bogdanov (housed at Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia).

Other sequences from GenBank were also used in the present study: 20 *A. draco*, four *A. latronum*, 40 *A. peninsulae*, and 11 *A. agrarius* (Table 1).

DNA ANALYSIS

DNA was extracted using the DNeasy Tissue Kit (Qiagen). A large portion of the cytochrome *b* gene (*cyt b*) was amplified using the universal polymerase chain reaction (PCR) primers L7 (5'-ACCAATGACATGAAAAATCATCGTT-3') and H6 (5'-TCTCCATTTCTGGTTTACAAGAC-3') (Kocher, Thomas & Meyer, 1989). The amplification reactions

were carried out in 50- μ L volumes including 12.5 μ L of each 2 μ M primer, 10 μ L of 1 mM dNTP, 5 μ L of 10 \times reaction buffer, 5 μ L of purified water and 0.25 μ L of 5 U μ L⁻¹ *Taq* DNA polymerase (Promega). Approximately 100 ng of DNA extract were used per PCR amplification. The amplifications were performed in a Labover PTC100 thermal cycler over 40 cycles (45 s at 94 °C, 45 s at 52 °C, and 2 min at 72 °C) with a final extension cycle of 10 min at 72 °C. The PCR products were purified. Both strands were sequenced using ABI 3700 automated DNA sequencer (Applied Biosystems) in accordance with the manufacturer's instructions.

The 118 new *Apodemus* sequences were deposited in the EMBL GenBank under accession numbers AM945740 to AM945857.

PHYLOGENETIC ANALYSIS

The published *cyt b* gene sequences for the studied *Apodemus* species were downloaded from GenBank (Table 1) and aligned to the new sequences using BIOEDIT, version 7.0.0 (Hall, 1999). The aligned sequences were analyzed using distances [Neighbour-joining (NJ); Saitou & Nei, 1987], maximum-parsimony (MP) (Fitch, 1971) using PAUP4b8 (Swofford, 2000), and the maximum likelihood (ML) criterion implemented in PHYML (Guindon & Gascuel, 2003). The most suitable models of DNA substitution for the distance and ML analyses were calculated using MODELTEST, version 3.0 (Posada & Crandall, 1998). MP analysis was conducted with the heuristic search algorithm, tree-bisection–reconnection (TBR) swapping and a maximum number of trees constrained to 1000. The robustness of the trees was assessed by bootstrap resampling (1000 random replications for NJ, MP, and ML analyses; Felsenstein, 1985).

PHYLOGEOGRAPHICAL AND GENETIC STRUCTURE ANALYSIS

A mismatch distribution of substitutional differences between pairs of haplotypes was calculated within each of the main populations of each species and compared with a fit to the Poisson model using DNASP, version 4.0 (Rozas *et al.*, 2003). This analysis provided an estimate of the population dynamics (either in recent expansion or rather stable in time) for the different lineages. Fu's F_s statistic (Fu, 1997) analyses were also used to detect population demographic expansion. The significance was evaluated by 1000 random permutations in ARLEQUIN, version 2.0 (Schneider, Roessli & Excoffier, 2000).

Haplotype (H_d) and nucleotide diversities (Nei, 1987) and their SD (Tajima, 1993) were estimated using DNASP, version 4.0.

Table 1. Map references, geographical locations, sample abbreviations, and GenBank Accession numbers of *Apodemus* haplotypes used in the present study

Map reference	Species	Geographic origin: country (city and/or province)		Total number of animals	Abbreviations	GenBank accession numbers (the associated studies are given in parenthesis)
	<i>Apodemus latronum</i>					
1		China	Lijiang, Yunnan	3	Yun	AB096836; AB096834; AB096835 (Suzuki <i>et al.</i> , 2003)
2			Yushu, Qinghai	4	Qinh	AM945829; AM945830; AM945831; AM945832 (present study)
3			Baoxing, Sichuan	1	Baox	AY389020 (Liu <i>et al.</i> , 2004)
4			Maerkang, Sichuan	15	Mar	AM945828; AM945823; AM945822; AM945820; AM945827; AM945826; AM945824; AM945833; AM945821; AM945825; AM945844; AM945834; AM945835; AM945836; AM945837 (present study)
				Total: 23		
	<i>Apodemus draco</i>					
1		China	Dali, Yunnan	5	Yun	AB109397; AB096833; AB096832; AB096831; AB096830 (Suzuki <i>et al.</i> , 2003)
2			Lijiang, Yunnan	3	Yun	AB096829; AB096828; AB096827 (Suzuki <i>et al.</i> , 2003)
3			Kuming, Yunnan	2	Yun	AB096826; AB096825 (Suzuki <i>et al.</i> , 2003)
4			Yunnan	3	Yun	AY389017; AY389018; AY389019 (Liu <i>et al.</i> , 2004)
5			Kuatun, Fujian	1	Est	AY389008 (Liu <i>et al.</i> , 2004)
6			Yaoluoping, Anhui	1	Est	AY389009 (Liu <i>et al.</i> , 2004)
7			Ninshan, Shaanxi	1	Shan	AY389004 (Liu <i>et al.</i> , 2004)
8			Shennongjia, Hubei	1	Hubei	AY389005 (Liu <i>et al.</i> , 2004)
9			Donlingshan, Beijing	1	Beijing	AY389006 (Liu <i>et al.</i> , 2004)
10			Baoxing, Sichuan	1	Baox	AY389010 (Liu <i>et al.</i> , 2004)
11			Emeishan, Sichuan	1	Emeis	AY389007 (Liu <i>et al.</i> , 2004)
12			Maerkang, Sichuan	13	Mar	AM945800; AM945801; AM945809; AM945810; AM945811; AM945812; AM945813; AM945814; AM945815; AM945816; AM945817; AM945818; AM945819 (present study)
13			Zhongguo, Sichuan	7	Zhong	AM945802; AM945803; AM945804; AM945805; AM945806; AM945807; AM945808 (present study)
				Total: 40		

Table 1. *Continued*

Map reference	Species	Geographic origin: country (city and/or province)		Total number of animals	Abbreviations	GenBank accession numbers (the associated studies are given in parenthesis)
1	<i>Apodemus peninsulae</i>	China	Rangtang, Sichuan	10	Rang	AM945779; AM945842; AM945786; AM945840; AM945789; AM945790; AM945791; AM945798; AM945799; AM945843 (present study)
2			Xiji, Ningxia	11	Ning	AM945792; AM945793; AM945794; AM945795, AM945796; AM945797; AM945780; AM945781; AM945841; AM945787; AM945788 (present study)
3			Baiyu, Sichuan	1	Baiy	AY389001 (Liu <i>et al.</i> , 2004)
4			Taishan, Shandong	1	Shand	AY389002 (Liu <i>et al.</i> , 2004)
5			Changchun, Jilin	1	Jilin	AY388999 (Liu <i>et al.</i> , 2004)
6			Haili, Heilongjiang	1	Haili	AY389000 (Liu <i>et al.</i> , 2004)
7			Baihaba, Xinjiang	4	Ping	AM945782; AM945783; AM945784; AM945785 (present study)
8	Korea	South Korea	4	Kor	AB073809; AB073810; AB073811; AB073818 (Serizawa <i>et al.</i> , 2002)	
9	Japan	Hayakita, Japan	4	Japan	AB032850 (Serizawa <i>et al.</i> , 2000); AB073790; AB073788; AB073789 (Serizawa <i>et al.</i> , 2002)	
10	Russia	Siberia	8	Sib	AB073806; AB073802; AB073807; AB073803; AB073805; AB073808; AB073801; AB073804 (Serizawa <i>et al.</i> , 2002)	
11		Primorye	12	Pri	AB073797; AB073796; AB073813; AB073794; AB073812; AB073800 ; AB07381 ; AB073799 ; AB073815; AB073816; AB073817; AB073798 (Serizawa <i>et al.</i> , 2002)	
12		Magadan	1	Mag	AB073795 (Serizawa <i>et al.</i> , 2002)	
13		Khabarovsk	4	Kha	AF427337; AF427338; AF427336; AF427335 (Dekonenko & Ivanov, Unpubl.)	
14		Sakhalin island	3	Sak	AB073793; AB073792; AB073791 (Serizawa <i>et al.</i> , 2002)	
				Total: 66		

Table 1. *Continued*

Map reference	Species	Geographic origin: country (city and/ or province)		Total number of animals	Abbreviations	GenBank accession numbers (the associated studies are given in parenthesis)
1	<i>Apodemus agrarius</i>	<i>Russia</i>	Novosibirsk	5	Nov	AM945851; AM945852; AM945853; AM945854; AM945848 (present study)
2			Chita, Transbaikal	1	Tch	AM945857 (present study)
3			Ussurisky region Primorye	1	Pri	AB032851 (Serizawa <i>et al.</i> , 2000);
4			Omsk region	1	Oms	AM945847 (present study)
5			Barnaul region	1	Altai	AM945838 (present study)
6			Khabarovsk region	5	Kha	AM945856; AM945855; AF427334; AF427333; AF427332 (present study)
7			Putyatin Island	4	Put	AM945773; AM945774; AM945849; AM945775 (present study)
8			Khorolsky district	3	Kho	AM945758; AM945759; AM945764 (present study)
9			Askold Island	2	Ask	AM945756; AM945762 (present study)
10			Reineke Island	5	Rei	AM945757; AM945763; AM945770; AM945771; AM945772 (present study)
11			Russky Island	5	Rus	AM945765; AM945766; AM945776; AM945777; AM945778 (present study)
12		<i>Korea</i>	Kanghwado Island, Korea	3	Kan	AM945767; AM945768; AM945769 (present study)
13			Hankor, Korea	2	Han	AY532796; AF264182
14			Korea	2	Kor	AM945760; AM945761 (present study)
15		<i>Kazakhstan</i>	East Kazakhstan	3	Kaz	AM945839; AM945845; AM945846 (present study)
16		<i>China</i>	Taishan, Shandong	1	Shand	AY389012 (Liu <i>et al.</i> , 2004)
17			Xi an, Shaanxi	1	Xia	AY389011 (Liu <i>et al.</i> , 2004)
18			Shanghai	1	Shang	AB096815 (Suzuki <i>et al.</i> , 2003)
19			Gansu	1	Gan	AM945753 (present study)
20			Tongzi, Guizhou	1	Ton	AB096809 (Suzuki <i>et al.</i> , 2003)
21			Xiji, Ningxia	16	Ning	AM945850; AM945748; AM945749; AM945750; AM945743; AM945740; AM945746; AM945747; AM945751; AM945752; AM945754; AM945742; AM945744; AM945741; AM945745; AM945755 (present study)
22			Taiwan	1	Taiw	AB096816 (Suzuki <i>et al.</i> , 2003)
				Total = 65		

Accession numbers were downloaded for the GeneBank database.

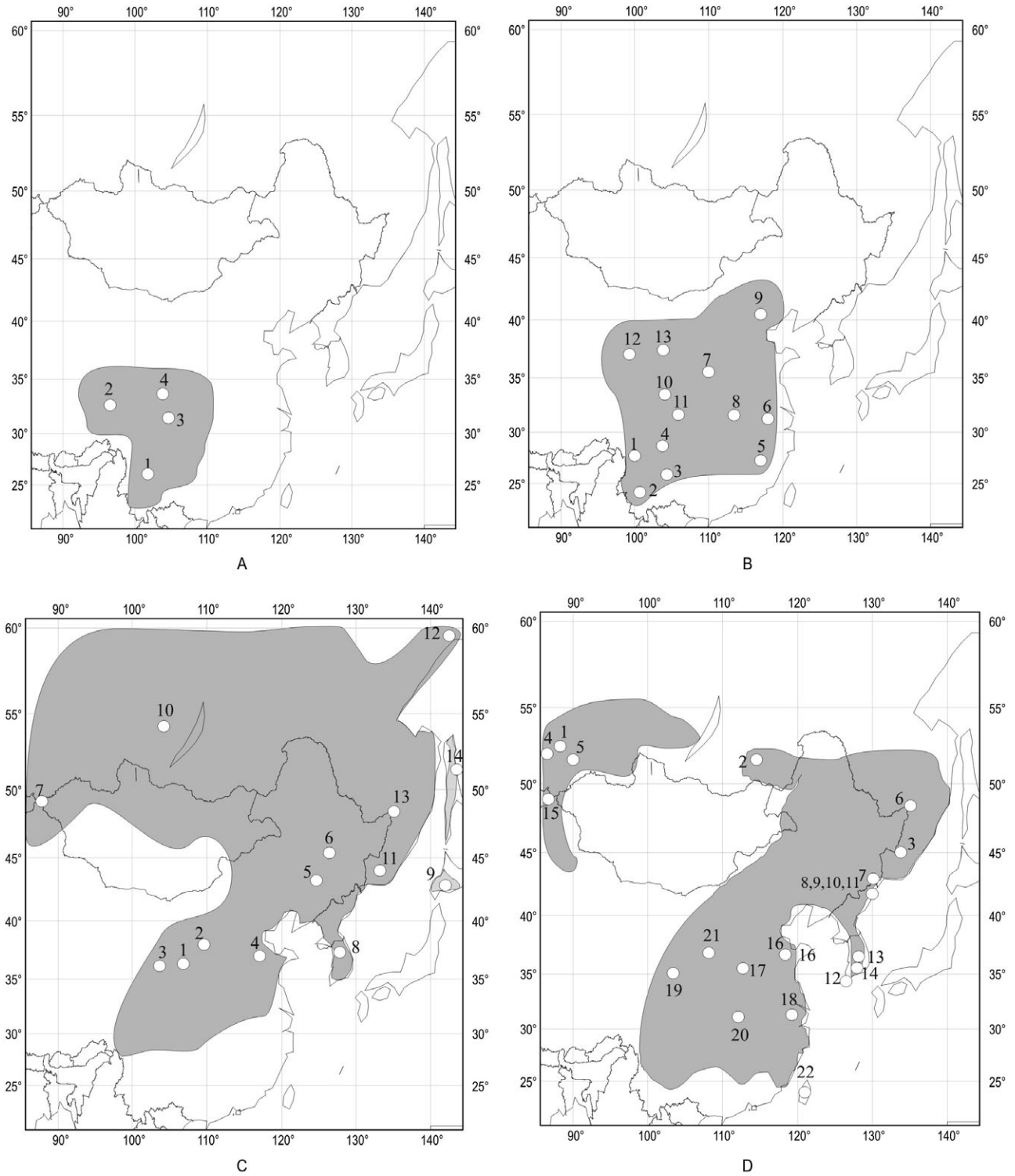


Figure 1. Geographic distribution of the *Apodemus* samples. A, *Apodemus latronum*. B, *Apodemus draco*. C, *Apodemus peninsulae*. D, *Apodemus agrarius*. The numbers correspond to the different sampled localities (Table 1).

Networks were constructed using minimum spanning network (MINSPNET in ARLEQUIN, version 2.0; Schneider *et al.*, 2000) and median-joining network (MJ) (Bandelt, Forster & Röhl, 1999; Network 4.000, available at <http://www.fluxus-engineering.com>) methods.

F_{ST} values were calculated with DNASP, version 4 (Rozas *et al.*, 2003) to analyse the genetic differentiation and estimate the gene flow among populations.

DIVERGENCE TIME

Relative-rate tests and an approximate time of divergence between the observed mitochondrial (mt)DNA lineages were calculated *sensu* Michaux *et al.* (2003). The genetic distance between two different lineages was corrected for ancestral mtDNA polymorphism using the formula of Edwards (1997). Two calibration points derived from paleontological data were used for this analysis. First, the divergence time between *A. mystacinus* and all the 'small' *Sylvaemus* was estimated at approximately 7 Mya (Aguilar & Michaux, 1996; Michaux *et al.*, 1997) and, second, the divergence between *A. sylvaticus* and *A. flavicollis* was estimated at approximately 4 Mya (Michaux & Pasquier, 1974; J. Michaux, pers. comm.).

RESULTS

SICHUAN FIELD MOUSE, *A. LATRONUM*

A total of 14 haplotypes were identified among the 23 *A. latronum* cytb sequences. Among the 1138 bp sequenced, 69 sites were variable and 44 were parsimony informative. The nucleotide frequencies were 32.6%, 24.7%, 12.5%, and 30.6% for A, C, G, and T, respectively.

The minimum spanning network (Fig. 2A) revealed five genetic lineages separated by at least four mutational steps: one corresponds to animals from the Yunnan province (southern China) (lineage 1), but also surprisingly from the Qinghai province (Yushu, southwestern China) (Figs 2A, 3A). The other lineages (lineages 2, 3, 4, and 5) correspond to woodmice from the Sichuan province (central China). These results are confirmed by the MJ network and the phylogenetic reconstructions performed using maximum parsimony algorithms, NJ and ML methods (not shown): the composition of the major lineages is identical to the one formed by the minimum spanning network (with a high bootstrap support in the range 72–82%).

To demonstrate potential regional refuge regions in China as suggested by other zoogeographic (Udvardy, 1969) and phylogeographic studies (Song *et al.*, 2009; Zhang *et al.*, 2009), we defined three groups within our dataset and tested whether they were characterized by high levels of nucleotide diversity as generally

observed in refugia. The first group corresponds to animals from Sichuan and is characterized by quite a high level of nucleotide diversity (π) (0.00576) compared to the second group of specimens from Yunnan (0.00387) and the third group of animals from Qinghai (0.00356) (Table 2). The intrapopulation genetic diversity follows the same pattern, with values of 0.6% K2P genetic divergence for the Sichuan population and 0.4%–0.3% for the Yunnan–Qinghai ones, respectively (Table 2).

By contrast, the Yunnan/Qinghai populations are separated by 1.5% of K2P genetic distance from the Sichuan ones (Table 3). This pattern is corroborated by high F_{ST} values among these populations (0.66), suggesting low gene flow among them (Table 4). However, these latter results must be considered with caution because the samplings for the Yunnan and Qinghai populations was low.

The mismatch distribution analyses (Fig. 4A) tends to a bell-shaped distribution for the Sichuan population, suggesting its sudden expansion. The Fu's F_s statistic confirmed a demographic expansion for this last population (Table 5). Animals from the Yunnan and Qinghai populations were not tested with these methods because the samplings were too low (three and four animals, respectively).

The divergence time between the Sichuan and Yunnan/Qinghai lineages was estimated to have taken place approximately 350 000 years ago.

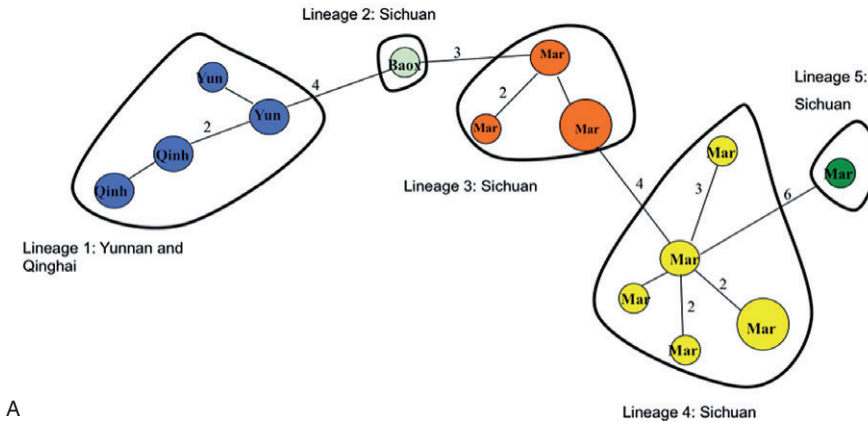
SOUTHERN CHINESE FIELD MOUSE, *A. DRACO*

A total of 22 haplotypes were identified among the 40 *A. draco* cyt *b* sequences analyzed. Of the 1135 bp sequenced, 204 sites were variable and 141 were parsimony informative. The nucleotide frequencies are 32.3%, 24.5%, 12.6%, and 30.6%, for A, C, G, and T, respectively.

The minimum spanning network (Fig. 2B) revealed six different lineages separated by 10 to 19 mutational steps. Two of them correspond to two populations from the Sichuan province (lineage 1 from Zhongguo and lineage 2 from Maerkang, Emeishan and Baoxing). Moreover, lineage 2 is divided into three sublineages separated by at least five mutational steps. Lineage 3 corresponds to animals from the Yunnan province (Dali, Kuming, and Lijiang) and is divided into four sublineages separated by four to nine mutational steps. Lineage 4 associates specimens from north-east China (Beijing and Hubei provinces), whereas lineages 5 and 6 correspond to specimens collected in eastern China (Kuatun, Fujian province, and Yaoluoping, Anhui province) (Figs 2B, 3B).

Similar to *A. latronum*, the MJ network and the phylogenetic reconstructions (not shown) also confirm

A. Latronum



A. draco

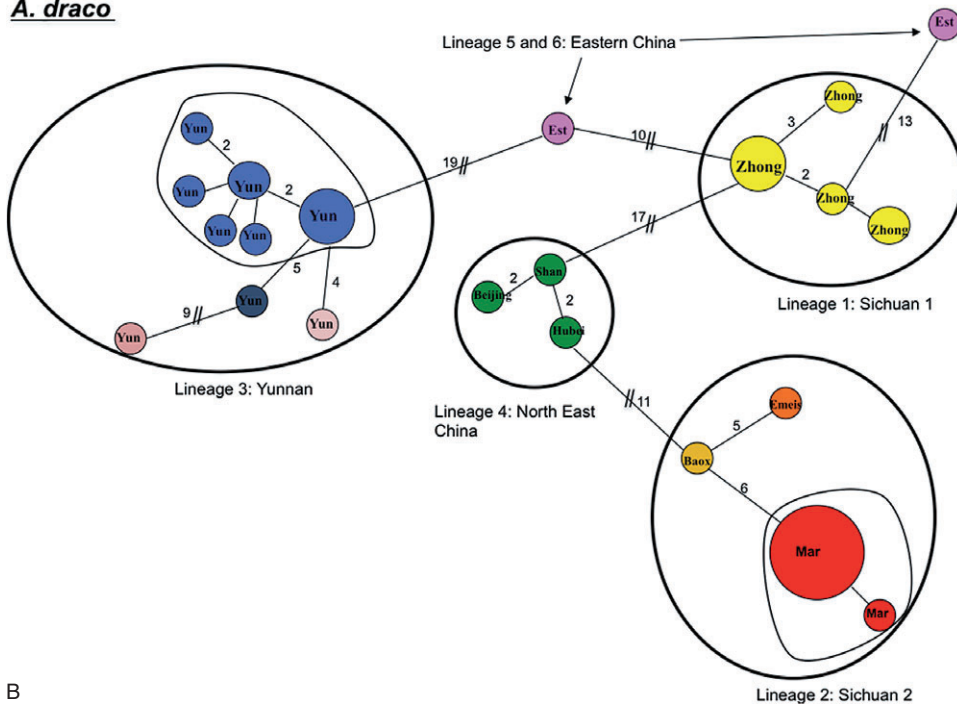


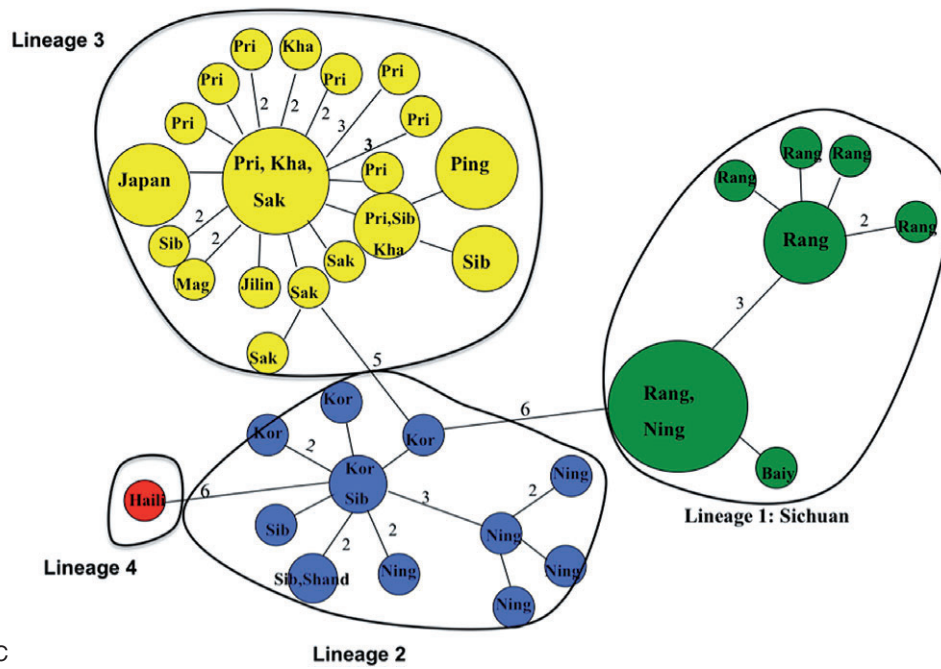
Figure 2. Minimum spanning networks of the mitochondrial DNA haplotypes demonstrated in the studied *Apodemus* species. A, *Apodemus latronum*. B, *Apodemus draco*. C, *Apodemus peninsulae*. D, *Apodemus agrarius*. The number of mutations (greater than one) between haplotypes is indicated in branches. Haplotype designations are provided in Table 1. Symbols correspond to the different genetic groups observed within each species (Fig. 3).

these complex results: the composition of the major lineages is identical to those formed by the minimum spanning network (with a high bootstrap support in the range 75–100%).

Again, to assess whether nucleotide diversity was higher within the potential regional refuge regions, we defined four groups within our dataset: a Sichuan group (associating lineages 1 and 2),

which showed a high level of nucleotide diversity compared to a second group including the animals from Yunnan (lineage 3), a third group of specimens from north-eastern China and a fourth one corresponding to the animals from eastern China (Table 2). The level of K2P genetic divergence gives similar results (Table 3). However, the results for eastern China must be taken with caution

A. peninsulae



A. agrarius

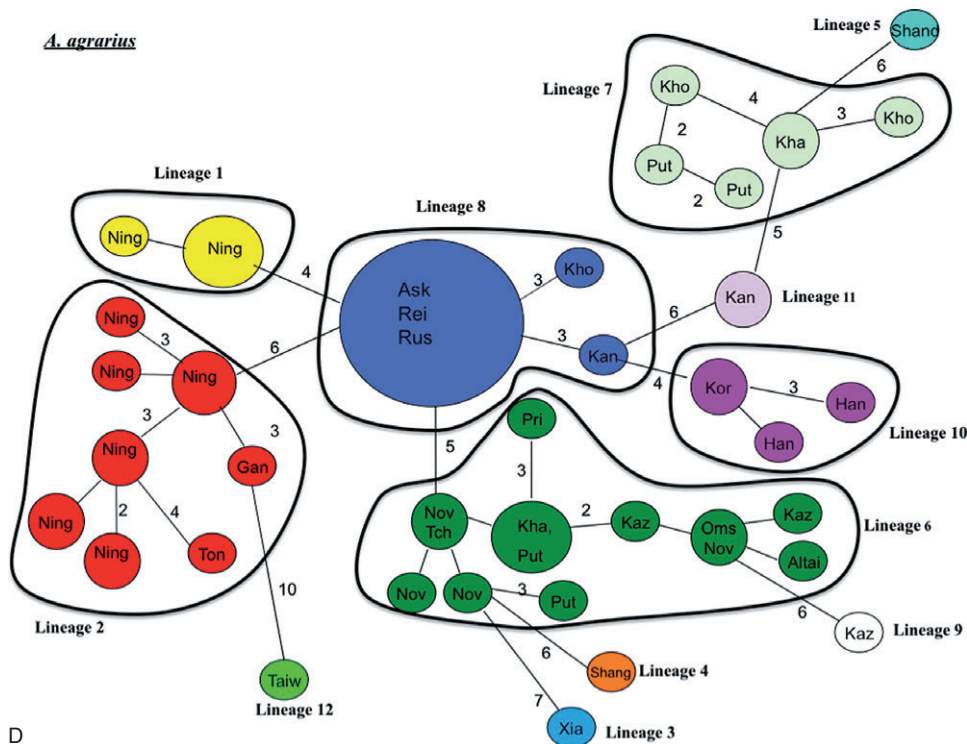


Figure 2. *Continued*

because the sampling for this region was very low ($N = 2$).

The mismatch distribution analyses (Fig. 4B) revealed a heterogeneous distribution for animals

from Sichuan (lineages 1 and 2) and Yunnan (lineage 3), suggesting that these populations are relatively stable. This analysis was not possible on the other lineages because of their low sampling.

Table 2. Genetic variability observed within the main groups of *Apodemus latronum*, *Apodemus draco*, *Apodemus peninsulæ*, and *Apodemus agrarius*

Species	Genetic groups	Number of samples	% Genetic diversity (K2P)	Number of haplotypes	$H_d \pm SD$	$\pi \pm SD$
<i>Apodemus latronum</i>	Yunnan (lineage 1)	3	0.4	3	0.857 \pm 0.102	0.00387 \pm 0.00070
	Sichuan (lineages 2 to 5)	16	0.6	10	0.933 \pm 0.040	0.00576 \pm 0.00089
	Qinghai	4	0.3	2	0.656 \pm 0.120	0.00356 \pm 0.00065
<i>Apodemus draco</i>	Sichuan (lineages 1 and 2)	22	1.2	9	0.814	0.03088 \pm 0.00473
	Yunnan (lineage 3)	13	1.3	10	0.949 \pm 0.051	0.01282 \pm 0.00276
	North-eastern China (lineage 4)	3	0.5	3	1 \pm 0.272	0.00575 \pm 0.00169
	Eastern China (lineages 5 and 6)	2	4.1	2	1 \pm 0.5	0.05179 \pm 0.02589
<i>Apodemus peninsulæ</i>	Sichuan, China (lineage 1)	17	0.8	10	0.875 \pm 0.070	0.00489 \pm 0.00046
	Ningxia, China	11	0.9	11	1 \pm 0.039	0.01477 \pm 0.00206
	Russian Far East	16	0.5	12	0.942 \pm 0.048	0.00636 \pm 0.00105
	Magadan, south Siberia and Altai	11	0.6	8	0.927 \pm 0.042	0.00876 \pm 0.00081
	Japan	4	0.0	3	0.833 \pm 0.222	0.00132 \pm 0.00048
	Sakhalin	3	0.2	3	1 \pm 0.272	0.00292 \pm 0.00048
<i>Apodemus agrarius</i>	China (lineages 1 to 5)	21	1.2	14	0.948 \pm 0.031	0.01144 \pm 0.00127
	Central Asia (lineages 6 and 9)	10	0.5	10	1 \pm 0.045	0.00553 \pm 0.00125
	Russian Far East (lineages 7 and 8)	26	0.9	10	0.772 \pm 0.078	0.00843 \pm 0.00117
	Korea (lineages 10 and 11)	7	0.9	6	0.952 \pm 0.096	0.00829 \pm 0.00151

H_d , haplotype diversity; π , nucleotide diversity.

The K2P genetic distances observed among the animals from the different regions were very high (average of 6.3%; Table 3). The F_{ST} values were also very high among the observed populations (Table 4).

The divergence time estimated between the main lineages corresponds to periods from 1.5–1.7 Myrs (north-eastern China/Sichuan) to 2.0–2.2 Myr (Sichuan/Yunnan).

KOREAN FIELD MOUSE, *A. PENINSULAE*

We identified a total of 38 haplotypes among the 66 *A. peninsulæ* cyt *b* sequences. Within the 1126 bp of the matrix, 208 sites (21%) were variable and 122 (12%) were parsimony informative. The nucleotide frequencies are 31%, 25.5%, 12.7%, and 30.8%, for A, C, G, and T, respectively.

The minimum spanning network (Fig. 2C) revealed three main lineages and another one separated by more than four mutational steps. Lineage 1 comprises animals from the Sichuan province (Baiyu and Rantang). Lineage 2 corresponds to northern (Ningxia province) and eastern Chinese (Shandong province), Korean, and Siberian animals (Fig. 3C). Lineage 3 is represented by the Russian Far East (Primorye, Khabarovsk, Sakhalin island, Magadan, Siberia), north-western (Xinjiang province) and north-eastern Chinese (Jilin province), as well as the Japanese specimens (Figs 2C, 3C). Finally, the fourth lineage

corresponds to an individual from the Heilongjiang province (north-eastern China).

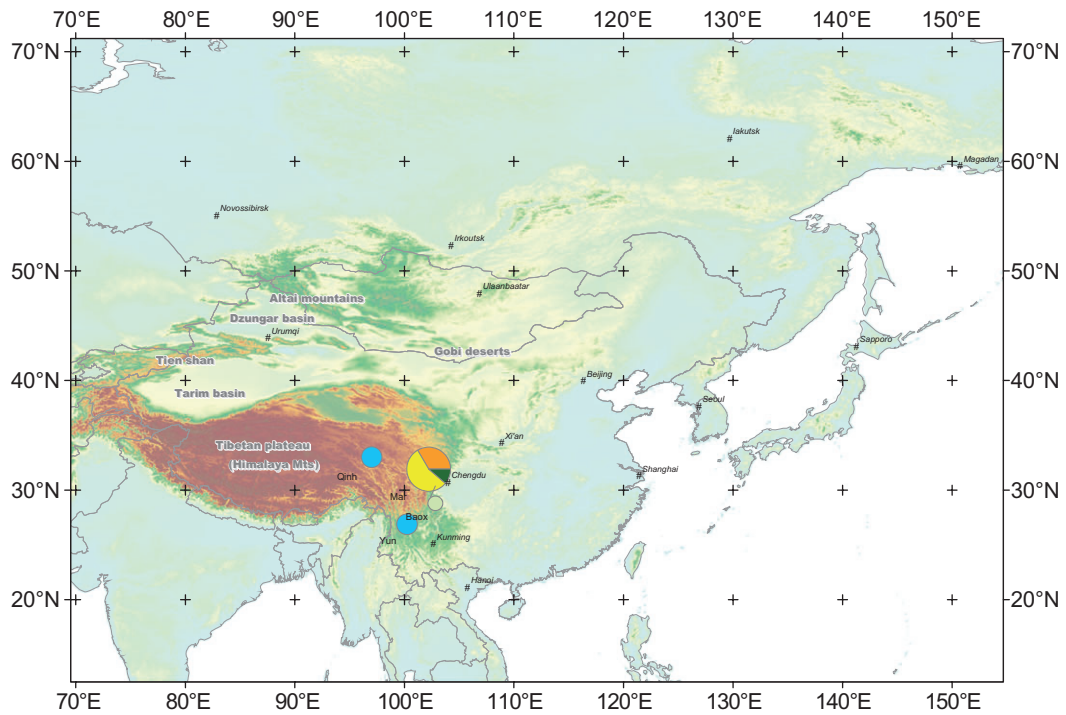
Again, the MJ network and the phylogenetic reconstructions (not shown) gave the same topology, and the major lineages were supported with a high bootstrap value in the range 86–99%.

As for two previous species, we defined six groups within our dataset to assess whether the nucleotide diversity was higher within the potential refuge regions: the first one corresponding to the Sichuan Chinese province, the second to specimens from the Ningxia Chinese province, the third to populations from the Russian Far East (Primorye, Khabarovsk), a fourth group of animals from the Sakhalin island, the fifth comprising the Japanese population, and, finally, the last one corresponding to animals from the Magadan region, southern Siberia, and the Altai region (Xinjiang province).

The animals from the Taishan (Shandong) region as well as from Korea were not taken into account in this analysis because the samplings were too low (one and four animals, respectively).

The Sichuan group as well as the animals from Japan and the Sakhalin Island have a relatively weak level of nucleotide diversity compared to the second group (Xinjiang) and the Siberian populations (Table 2). The animals from the Russian Far East (group 3), southern Siberia, and the Altai region (group 6) showed intermediate values

A *A. latronum*



B *A. draco*

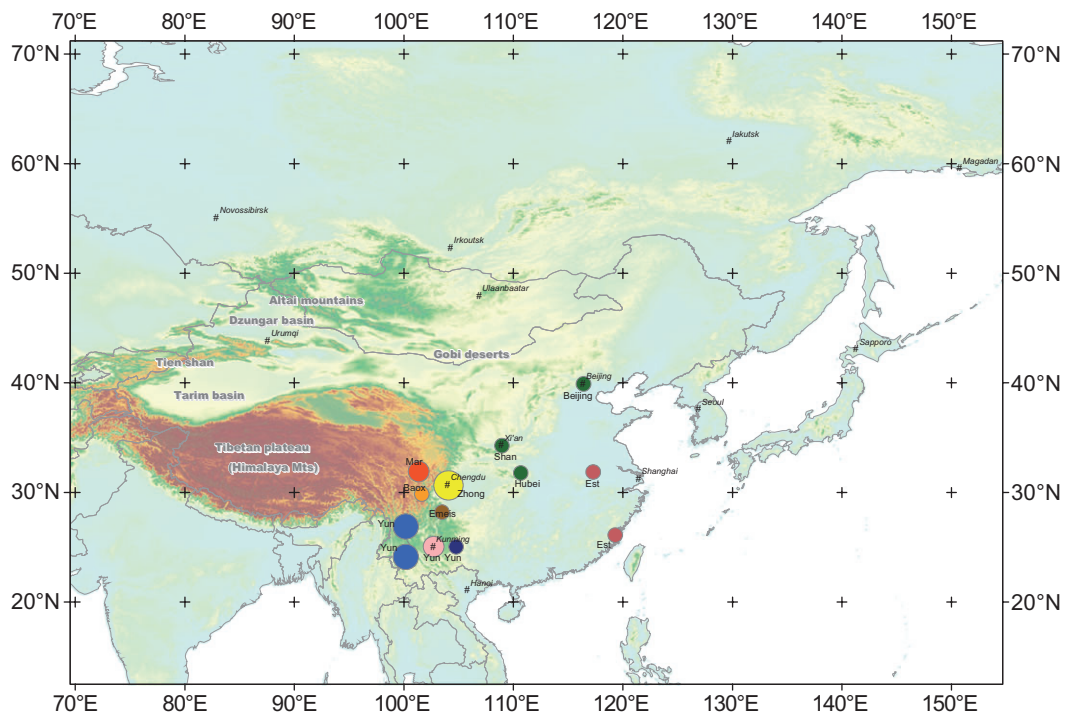
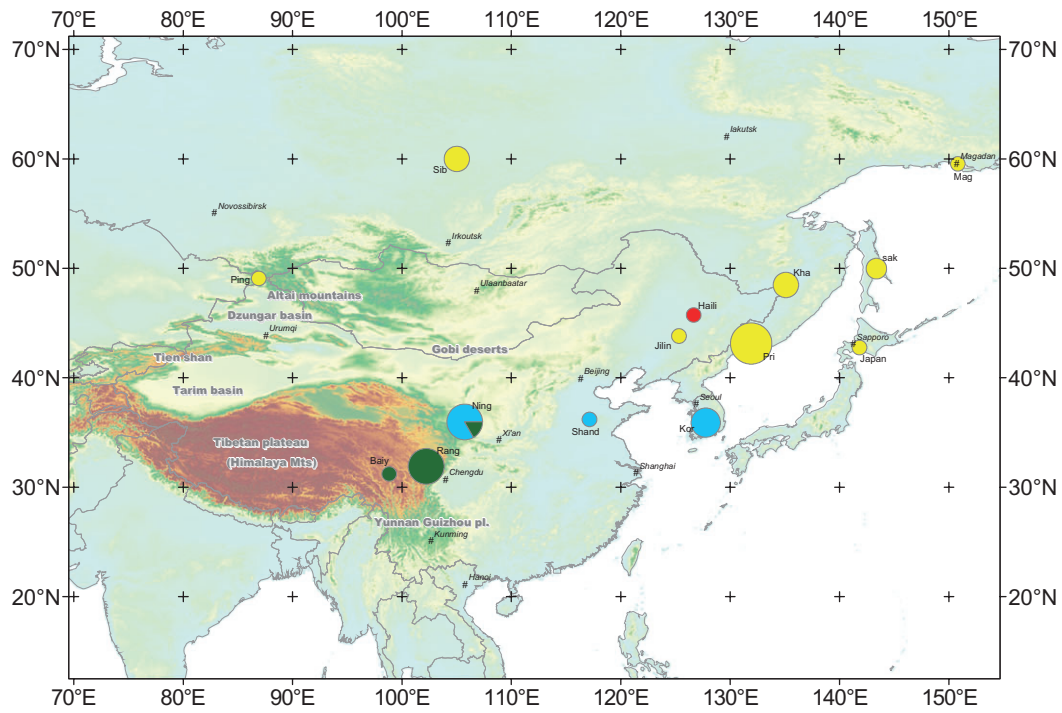


Figure 3. Geographic distribution of the different genetic lineages observed on the studied *Apodemus* species. A, *Apodemus latronum*. B, *Apodemus draco*. C, *Apodemus peninsulae*. D, *Apodemus agrarius*. The genetic lineages are described according to the colours given in Fig. 2. The codes of the different sampling localities are given according to Table 1 and Fig. 2. The names of the main cities and biogeographical barriers are also shown.

C *A. peninsulae*



D *A. agrarius*

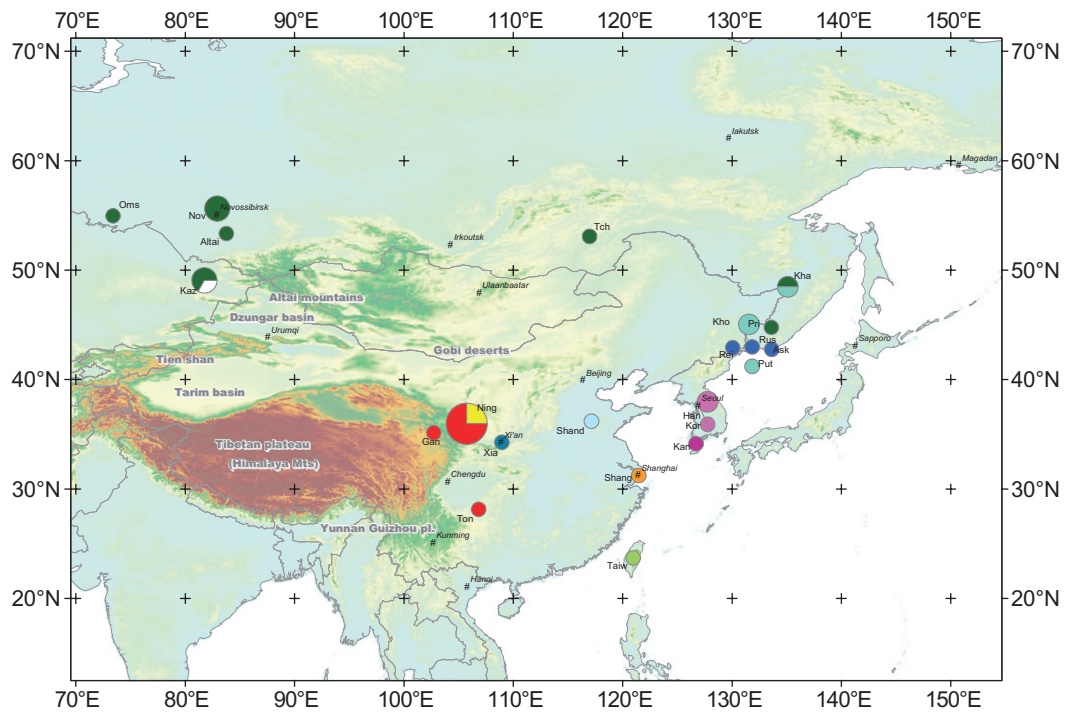


Figure 3. Continued

Table 3. Genetic divergences (K2P distances) between the main groups of *Apodemus latronum*, *Apodemus draco*, *Apodemus peninsulae*, and *Apodemus agrarius*

<i>Apodemus latronum</i>	Sichuan				
Yunnan/Qinhai	0.015/0.010				
<i>Apodemus draco</i>	Yunnan (lineage 3)	Sichuan (lineages 1 and 2)	North-eastern China (lineage 4)		
Sichuan (lineages 1 and 2)	0.064/0.055				
North-eastern China (lineage 4)	0.061/0.052	0.051/0.032			
Eastern China (lineages 5 and 6)	0.064/0.037	0.043/0.029	0.056/0.032		
<i>Apodemus peninsulae</i>	Japan	Russian Far East	Magadan, southern Siberia and Altai	Sakhalin island	Ningxia (China)
Russian Far East	0.006/0.003				
Magadan, southern Siberia and Altai	0.009/0.006	0.007/0.001			
Sakhalin island	0.005/0.004	0.005/0.001	0.008/0.003		
Ningxia (China)	0.023/0.019	0.024/0.017	0.027/0.019	0.023/0.017	
Sichuan (China, lineage1)	0.037/0.033	0.036/0.029	0.039/0.032	0.031/0.026	0.033/0.025
<i>Apodemus agrarius</i>	Central Asia (lineages 6 and 9)	China (lineages 1 to 5)	Russian Far East (lineages 7 and 8)	Taiwan (Lineage 12)	
China (lineages 1 to 5)	0.015/0.007				
Russian Far East (lineages 7 and 8)	0.012/0.005	0.014/0.003			
Taiwan (lineage 12)	0.023/0.021	0.019/0.013	0.022/0.018		
Korea (lineages 10 and 11)	0.013/0.006	0.014/0.004	0.011/0.003	0.023/0.018	

The two values correspond to the P_{mean} and P distances.

(Table 2). The levels of K2P genetic divergence are summarized in Table 2. They once again demonstrated that the Sakhalin and Japan populations are characterized by a weak level of genetic diversity. However, this result must be taken with caution because the samplings for these regions were low (three and four animals, respectively). By contrast, the Sichuan animals and specimens from Ningxia (China) showed higher values (0.8–0.9% K2P distances).

The mismatch distribution analyses (Fig. 4C) revealed that the population from Sichuan (lineage 1) was stable. This result was corroborated by Fu's F_S analyses. By contrast, mismatch distribution and Fu's F_S analyses demonstrated that animals from Ningxia (China), as well as from the Russian Far East and the Magadan-Siberia-Altai region, presented a signal of expansion (Table 5).

A high value of F_{ST} (in the range 0.57–0.81) was obtained between the main groups, suggesting a weak gene flow between these regions. However, F_{ST} is low

between the Russian Far East animals and those from Sakhalin (0.25915) or the populations from Siberia-Magadan-Altai, suggesting recent contacts between these regions (Table 4).

The divergence time estimated between the main lineages corresponds to relatively recent periods, in the range 105 000–115 000 years (Japan/Russian Far East) to 140 000–150 000 years (Sakhalin/Russian Far East).

STRIPED FIELD MOUSE, *A. agrarius*

A total of 37 haplotypes among the 65 *A. agrarius* *cytb* sequences were identified. Within the 1113 bp of the matrix, 141 sites (13%) were variable and 68 (6.1%) were parsimony informative. The nucleotide frequencies are 30.1%, 27.2%, 13.4%, and 29.3%, for A, C, G, and T, respectively.

The minimum spanning network (Fig. 2D) of *A. agrarius* showed an important genetic structure with twelve lineages separated by at least four muta-

Table 4. Genetic differentiation (F_{st} values) between the main groups of *Apodemus latronum*, *Apodemus draco*, *Apodemus peninsulae*, and *Apodemus agrarius*

<i>Apodemus latronum</i>	Sichuan				
Yunnan/Qinhai	0.66489				
<i>Apodemus draco</i>	Yunnan (lineage 3)	North-eastern China (lineage 4)	Eastern China (lineages 5 and 6)		
North-eastern China (lineage 4)	0.8623				
Eastern China (lineages 5 and 6)	0.57915	0.57143			
Sichuan (lineages 1 and 2)	0.68958	0.56922	0.26229		
<i>Apodemus peninsulae</i>	Japan	Sakhaline island	Russian Far East	Magadan, south Siberia and Altai	Ningxia (China)
Sakhaline island	0.800				
Russian Far East	0.57931	0.25915			
Magadan, southern Siberia and Altai	0.64138	0.44474	0.17644		
Ningxia (China)	0.81039	0.75965	0.70753	0.71764	
Sichuan (China, lineage 1)	0.89171	0.83666	0.81910	0.77287	0.74707
<i>Apodemus agrarius</i>	Central Asia (Lineage 6 and 9)	China (Lineage 1 to 5)	Russian Far East (Lineage 7 and 8)		
China (lineages 1 to 5)	0.43285				
Russian Far East (lineages 7 and 8)	0.38708	0.22033			
Korea (lineages 10 and 11)	0.46303	0.26092	0.22039		

Table 5. Fu's F_s values for the main groups of *Apodemus latronum*, *Apodemus draco*, *Apodemus peninsulae*, and *Apodemus agrarius*

Species	Geographic groups	Fu's F_s statistic	P	Demographic signal
<i>Apodemus latronum</i>	Sichuan	-2.101	0.071	Expansion
<i>Apodemus draco</i>	Sichuan	1.51	0.196	Stable
	Yunnan	-1.035	0.174	Stable
<i>Apodemus peninsulae</i>	Sichuan, China (lineage 1)	-1.538	0.104	Stable
	Ningxia, China	-6.425	0.002	Expansion
	Russian Far East	-8.562	0	Expansion
	Magadan, south Siberia and Altai	-6.325	0.005	Expansion
<i>Apodemus agrarius</i>	China (lineages 1 to 5)	-1.103	0.166	Stable
	Central Asia (lineages 6 and 9)	-6.292	0.002	Expansion
	Russian Far East (lineages 7 and 8)	0.838	0.169	Stable
	Korea (lineages 10 and 11)	-0.354	0.304	Stable

The lineages characterized by a too low sampling were not tested using this analysis.

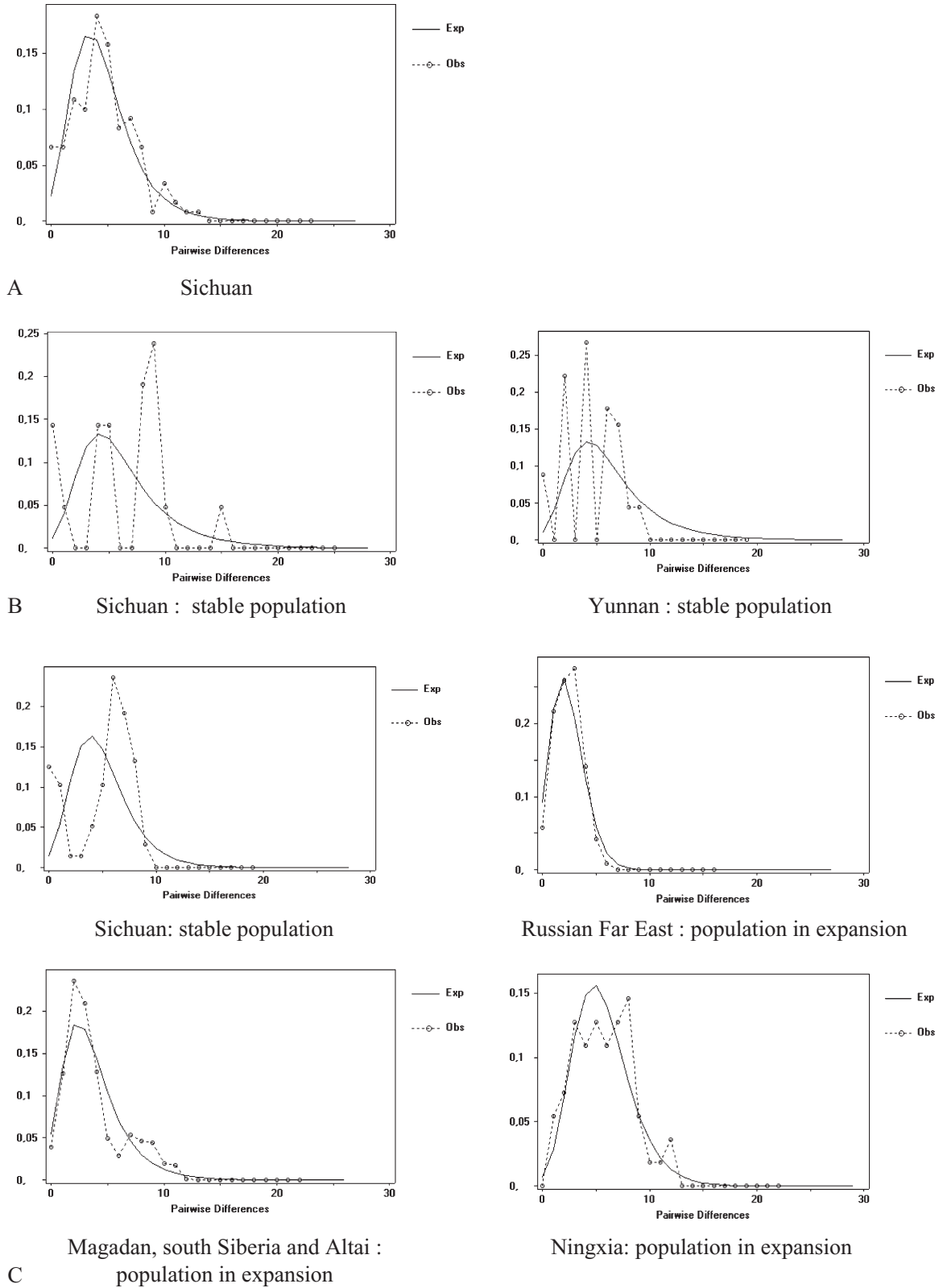


Figure 4. Mismatch distribution analysis for the main lineages evidenced in the studied *Apodemus* species. A, *Apodemus latronum*. B, *Apodemus draco*. C, *Apodemus peninsulae*. D, *Apodemus agrarius*. Lineages represented by too few specimens ($N < 10$) were not tested using this method.

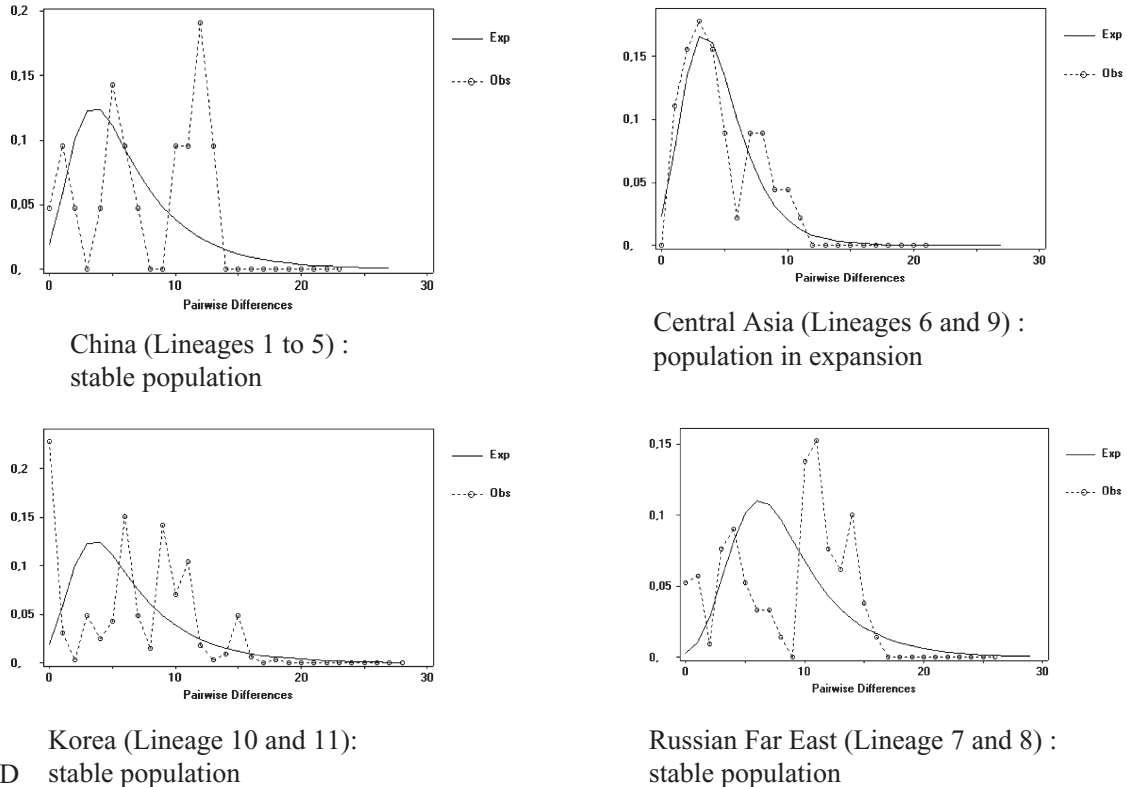


Figure 4. *Continued*

tional steps. The Chinese specimens are spread in five different genetic lineages distributed all over the country (Fig. 3D): central (Shaanxi, Ningxia, Gansu provinces) and southern (Guizhou province) China (lineages 1, 2 and 3) and eastern China (Shandong province, Shanghai) (lineages 4 and 5).

The Chinese lineages do not form a particular monophyletic group but are rather dispersed all over the network, where they are connected to the Russian Far East haplotypes. This suggests close relationships between populations from these two regions.

The individuals from Russia and Kazakhstan are distributed in three main lineages (lineages 6, 7 and 8). With the exception of one individual from Kazakhstan (lineage 9), which appears to be genetically differentiated, no particular geographic structure can be observed within and among these lineages: all the populations appear to be mixed (Fig. 3D). The animals from Central Asia and Kazakhstan are strongly linked to populations from the Russian Far East and west Siberia (lineage 6) (Figs 2D, 3D). This result suggests a closer relationship between animals from these regions than for those from China or Korea.

Korean animals are split into two genetic lineages (lineages 10 and 11), also suggesting an important genetic diversity.

The MJ network and the phylogenetic reconstructions (not shown) gave the same topology, and the major lineages were supported with a high bootstrap value in the range 88–100%.

Four main groups were defined within our dataset to analyse the genetic diversity within the potential refuge regions: China, Korea, Russian Far East, and Central Asia. The Chinese populations are characterized by the highest level of nucleotide diversity in contrast to the populations of Central Asia, and Kazakhstan presenting the lowest values of π (Table 2). Animals from the Russian Far East and Korea gave intermediate values. The levels of K2P genetic divergence within each of these population groups gave similar tendencies (Table 2). The values of this last index were quite similar among these population groups (between 1.2–1.5% of K2P distance) (Table 3). However, the sample from Taiwan (lineage 12) appears to be slightly more differentiated from all the others, with values in the range 1.9–2.3% K2P distance. However, these data must be interpreted with caution because the sampling for this region was very low ($N = 1$).

The mismatch distribution analyses revealed that the Chinese (lineages 1 to 5), Korean (lineages 10 and 11) and Russian Far East (lineages 7 and 8) populations are stable (heterogeneous pattern; Fig. 4D). By

contrast, animals originating from west Siberia and Kazakhstan (lineages 6 and 9), presented a bell-shaped distribution, suggesting a sudden expansion. Fu's F_s index (Table 5) corroborates these patterns.

The separation between Chinese–Russian Far East–Korean animals and central Asian animals is estimated to have taken place approximately 175 000–192 000 years ago. The Taiwan population appears to have been isolated from the Chinese continental populations for 450 000–500 000 years.

DISCUSSION

A COMPLEX PHYLOGEOGRAPHIC STRUCTURE ASSOCIATED WITH THE QUATERNARY PERIOD

The present study clearly demonstrated the existence of an important geographic structure of the genetic variability within each of the studied species. According to the divergence time analyses, this genetic differentiation could have occurred during the Quaternary. Therefore, although a great part of China was not covered by continental ice sheets during the Quaternary cold phases (Axelrod, Ai-Shehbaz & Raven, 1996; Zhou, 1997; Zhou *et al.*, 2004), it is probable that the cold and dry climates, which nevertheless characterized these last periods (Harrison *et al.*, 2001), played an important role in the isolation of field mice populations in different regions of China and the Russian Far East. This later led to the appearance of different genetic lineages through allopatric differentiations. This scenario is similar to that generally proposed for other regions, such as Europe or Northern America, to explain the intraspecific genetic structure of many organisms (Avice, 2000). This would also explain the genetic structure of the few other mammal species that were studied in the Asian Far East regions (Mahmut *et al.*, 2002; Li *et al.*, 2005; Oshida *et al.*, 2005), as well as of the birds (*Phasianus colchicus* and *Alcippe morrisonia*: Qu *et al.*, 2009; Song *et al.*, 2009), amphibians (*Pelophylax nigromaculata*; Zhang *et al.*, 2008) or plants (*Dysosma versipellis*; Qiu *et al.*, 2009).

However, compared to the other continents, these isolation processes have probably been enhanced by the particular complex geology and topography of China and the Russian Far East. Indeed, different paleoecological and geological data (Harrison *et al.*, 2001; Rousseau D. D., pers. comm.) indicate that the uplift of the Tibetan plateau led to the broadening of several desert regions (such as the Gobi desert) during the Quaternary cold phases (Chen *et al.*, 2008). For example, this phenomenon resulted in the repeated isolation of the Russian Far East and the Korean peninsula. According to the results of the present study, this would have led to the isolation of some populations of *A. peninsulae* and *A. agrarius*

within this last region. The complicated topology of southern China, and particularly the existence of deep river valley systems (Zhang *et al.*, 2009) (Fig. 3), also probably led to the isolation and the differentiation of several genetic lineages of field mice, as already suggested for other species such as the bird *A. morrisonia* (Song *et al.*, 2009).

The combination of these historical and geological processes would therefore be at the origin of the very complex phylogeographic structure of the Asian Far East field mice populations (e.g. 12 different genetic lineages within *A. agrarius*).

Although each species seems to give a particular phylogeographic pattern, some general trends seem to appear. First, populations of *A. draco* and *A. latronum* from the Yunnan (southern China) appear to be highly divergent from those from the other regions (Fig. 3A, B). Second, the same trend is also observed for the populations from the Sichuan (central China) (*A. draco*, *A. latronum*, and *A. peninsulae*) or the eastern part of China (*A. draco*, *A. agrarius*) (Fig. 3). Finally, particular lineages appear to be also present in *A. agrarius* and *A. peninsulae* from the Russian Far East (Fig. 3).

These results suggest that the above regions could correspond to refuge areas for the field mice. Other factors appear to corroborate this hypothesis. Indeed, even if at least three glaciations were detected in the south-western Chinese Plateau (comprising the Sichuan and Yunnan), the ice cover could have been permanent during these periods only in the higher altitude and central regions of the Tibetan Plateau (Shi, Zheng & Li, 1990). Therefore, some areas located in the eastern margin of this plateau were free of ice cover or less covered by ice (Shi, 1996) and would have provided suitable refugia for temperate species. Alpine meadow and grassland could have extended in these regions during the extensive glaciation periods (Liu *et al.*, 2004). This is probably why many relict floras of the Tertiary still survive in these areas that are considered as a refuge for temperate and subtropical floras (Zheng & Liu, 1995). Moreover, the eastern regions were also proposed as refuges for temperate and tropical–subtropical faunas during the Quaternary cold stages (Zhang, 2004; Zhang *et al.*, 2009). For example, pikas (*Ochotona* spp.) survived in these regions (Li, 1986), providing indirect evidence for the occurrence of eastern refugia.

According to the present data, two main phylogeographic patterns can be observed among the four studied species of field mice:

- A phylogeographic history of Chinese endemics. This pattern is observed in *A. latronum* and *A. draco*. Indeed, their particular ecological habits, adapted to subtropical or temperate forests appear

to have allowed these species to colonize neither the western mountainous Chinese regions, nor north-east China, nor the Russian Far East. Therefore, they appear to have evolved for several million years in regions covering central, southern, and eastern China.

- A phylogeographic history covering not only China, but also the Russian Far East, southern Siberia, and Central Asia. This pattern is observed in *A. agrarius* and *A. peninsulae*. Indeed, their more generalist ecology, adapted to forest but also open habitats (steppe regions), probably allowed these species to colonize broader distributions areas and survive there.

These two patterns will be developed below.

PHYLOGEOGRAPHY OF ENDEMIC CHINESE SPECIES (*A. LATRONUM* AND *A. DRACO*)

Apodemus draco

Six main lineages widespread in different Chinese regions (Sichuan, Yunnan, Beijing region, and the Eastern Pacific coast) were demonstrated in *A. draco* (Fig. 3B). This complex phylogeographic structure is probably the result of past population fragmentations all over China, which could again be associated with early Quaternary glaciations. Indeed, the divergence time analyses gave an estimation of at least 1.5 Mya for the separation of the different lineages. The genetic differentiation of this species would therefore be much older than that of the other Chinese *Apodemus* species.

The isolation of these lineages in different refuges that were probably localized in the south-western Chinese plateau and eastern China could have been enhanced by the presence of deep river valleys (Chang Jiang, Yalong Jiang, Wu Jiang) and the Hengduan Mountains (Li & Su, 1996) of southern and western China. Indeed, these could have acted as a barrier to gene flow for this species. The west–east boundary found in the present study also appears to exist for other organisms dispersed over China such as plants (e.g. *D. versipellis*; Qiu *et al.*, 2009), birds (e.g. *P. colchicus*; Qu *et al.*, 2009) or amphibians (e.g. *P. nigromaculata*; Zhang *et al.*, 2008). These species would have experienced the same continuous cooling periods and gene flow barrier through the Pleistocene.

The Yunnan-Guizhou plateau underwent gradual uplifting during the last million years, exhibiting topographical complexity and a variety of elevations and climates, ranging from tropical to arctic (Suzuki *et al.*, 2003). Therefore, these particular topographies and climates may also have contributed to isolate some populations of *A. draco*, leading to the differentiation of particular lineages in this region (Fig. 3B). Such a scenario was already proposed to explain

the complex genetic differentiation observed on the montane frog *Leptobranchium ailaonicum* (Zhang *et al.*, 2009).

The existence of two highly differentiated lineages in eastern coastal China (Fig. 3B) could be explained by the cyclic arid phases of the climate and the extension of the desert to the Pacific regions during the Quaternary cold phases (Zhou *et al.*, 2004; Chen *et al.*, 2008). These would have led to the isolation of populations in different coastal refuges, which later led to the differentiation of several *A. draco* genetic lineages. Indeed, eastern China is generally considered as a territory having moister and warmer climates, even during the Quaternary cold phases (Zhang *et al.*, 2008). Such eastern refuges were also proposed for the black spotted frog (*P. nigromaculata*) (Zhang *et al.*, 2008).

Finally, the isolation of the two Sichuan groups of *A. draco* from Zhongguo, Maerkang, and Baoxing (Fig. 3B) could be explained by the presence of deep river valleys isolating these Sichuan regions (Zhang *et al.*, 2008). This isolation would have been particularly important during the Quaternary glaciations. This hypothesis of past isolation is corroborated by the divergence data analysis, which suggests a separation of the Sichuan populations from the eastern ones at least 1.5 Mya.

Apodemus latronum

The phylogeographic pattern observed in *A. latronum* demonstrated strong differentiated populations in the Sichuan and Yunnan regions (Fig. 3A). Similar to *A. draco*, this result could be explained by the presence of many refuges in the south-western Chinese Plateau (Song *et al.*, 2009; Zhang *et al.*, 2009), which would have played an important role for the genetic differentiation of this *Apodemus* species. This hypothesis was already set forth to explain the important geographical structure of the tree *Juniperus przewalskii* (Cupressaceae) endemic to the Tibetan Plateau (Zhang *et al.*, 2005).

Moreover, the animals from Yushu (Qinghai province) appeared to be strongly linked to the Yunnan ones (Fig. 3A), suggesting gene flow between these two regions, although they are separated by several hundred kilometres. This suggests that field mice could cross these mountainous regions, probably through the valleys systems (e.g. the Chang Yangtze River).

Similar to *A. draco*, the high level of nucleotide diversity observed in Sichuan and Yunnan suggests that these regions could represent a refuge for *A. latronum*. This result is corroborated by the mismatch distribution analysis suggesting that the populations from Yushu Qinghai and Yunnan are stable. It reinforces the hypothesis of the south-western

Chinese region as an important refuge for many organisms (Zheng & Liu, 1995; Zhang, 2004; Zhang *et al.*, 2009). This information is of prime interest from a conservation point of view.

PHYLOGEOGRAPHY OF THE ASIAN FAR EAST SPECIES:
A. AGRARIUS AND *A. PENINSULAE*

Apodemus agrarius

Apodemus agrarius showed a very complex genetic structure in the studied regions. China, the Russian Far East, and Korea appear to represent important centres of diversification for this species because no less than 12 different genetic lineages were found within these regions.

As suggested by the divergence time analyses, this diversification could be the result of the isolation of several populations during the Quaternary ice ages leading to allopatric differentiation processes.

The cooler climate periods allowed the extension of the Gobi desert to the Pacific areas and probably led to the isolation of the Russian Far East (Primorye and Khabarovsk regions), as well as of the Korean peninsula populations (Harrison *et al.*, 2001; Zhou *et al.*, 2004; Zhang *et al.*, 2008). This last region, characterized by south temperate mountain climate, was not deeply affected by the global Quaternary climate changes (Liu & Li, 1996) and therefore could have allowed many organisms to survive, even during the coldest phases. Some morphological (Jones & Johnson, 1965) and genetic studies (Koh *et al.*, 1999) have suggested that the striped field mice from the Korean peninsula could be differentiated from the other Far East populations, and they were proposed as two particular subspecies: *Apodemus agrarius pallescens* in South Korea and *Apodemus agrarius corea* in central Korea. The results of the present study tend to confirm this hypothesis because the studied animals appear to be isolated in two different lineages separated by at least four mutational steps (Fig. 2D). According to Zhou *et al.* (2004), the extension of the arid zones during the ice ages was probably linked to an important discordant state of the mode of monsoons. Such an isolation of populations from the Korean peninsula was also observed on the black spotted frog (*Pelophylax nigromaculata*) (Zhang *et al.*, 2008).

The Russian Far East could have also been a potential refuge for *A. agrarius*. This result is corroborated by the high values of genetic, haplotype, and nucleotide diversities (Table 2), as well as by the mismatch distribution and Fu's F_S indices.

The same tendencies (Table 2) were observed in China, suggesting that this region was probably also a refuge for the striped field mouse during the Quaternary glaciations, similar to *A. draco* and *A.*

latronum. This result is corroborated by the mismatch distribution and Fu's F_S analyses suggesting stable *A. agrarius* populations that did not suffer from recent bottlenecks within this region. However, our sampling does not allow to locate the Chinese refuges precisely and clearly. These hypotheses follow the results obtained in other studies carried out on the Asian *Apodemus* species (Suzuki *et al.*, 2003).

Recent fragmentation of A. agrarius

The distribution area of *A. agrarius* is divided into two main and geographically well isolated parts. The first one corresponds to China and the Russian Far East (group 1) and the other comprises a large region, starting from the western part of the Baikal Lake to Central Europe (group 2).

There are important questions concerning the genetic relationships between populations of these two distribution ranges: Are these populations genetically related with frequent gene flow between them or have they been separated for a long time? What is the region of origin of *A. agrarius*? What are demographic histories of these population groups?

Our results demonstrate a closer relationship between the central-western Palearctic populations (group 2) and those from the Russian Far East compared to animals from China (Fig. 3D). Moreover, the levels of nucleotide and haplotype diversities are significantly higher in group 1 compared to populations from group 2. This result is similar to others previously obtained using proteins and the random amplification of polymorphic DNA-PCR method (Atopkin, Bogdanov & Chelomina, 2007). Demographic parameters (mismatch distribution analyses, Fu's F_S indices) also show that group 1 is characterized by a signal of stable populations, whereas group 2 corresponds more to populations that have recently expanded.

These combined data lead to the following scenario for the evolutionary history of *A. agrarius*: this species appeared approximately 5 Mya (Michaux *et al.*, 2002; Suzuki *et al.*, 2003) in the Asian Far East and probably in China (higher number of genetic lineages, higher genetic and nucleotide diversities, signal of stable populations). Subsequently, it could have colonized other extreme East Asian areas, such as Ussuriland (Russian Far East) (important relationships between populations of these two regions). Approximately 175 000–190 000 years ago, *A. agrarius* was able to colonize the Central Palearctic region, probably via Russian Far East populations. Indeed, the network demonstrates closer relationships between these latter populations. Moreover, as observed in several other organisms (Stakel, 1998), a western colonization via the Chinese striped field mice is very unlikely because this region is sur-

rounded by important biogeographic barriers, such as the Himalayan Mountains or the Gobi desert (Fig. 3D).

Finally, the low levels of genetic and nucleotide diversities as well as the signal of recent expansion observed in the mismatch distribution analysis, suggest that the central–western Palearctic populations expanded relatively recently.

This scenario must however be confirmed by studies on larger sets of specimens, particularly from the western part of the striped field mouse distribution area.

Phylogeographic structure of A. peninsulae

The results obtained demonstrate an important level of genetic diversity for the Chinese populations (two main lineages, high levels of genetic and nucleotide diversities). Similar to the other *Apodemus* species, this result suggests that China was a refuge for *A. peninsulae*.

The Korean populations appear to be closely related to the central Chinese animals, suggesting frequent gene flow between populations from these regions (Fig. 3C). By contrast, the Korean animals appear to be highly differentiated from the Russian Far East populations (Fig. 3C). This latter result is in agreement with previous molecular (Serizawa *et al.*, 2002) and paleogeographical data (Tsukada, 1985; Nazarenko, 1990) and is quite surprising because these last two regions are geographically very close compared to China. The study of a larger number of specimens from this area will probably help to better understand this particular genetic structure.

As already observed for *A. agrarius*, Eastern Russia and Siberia could also have been refuge areas for *A. peninsulae*. This result is corroborated by the high values of nucleotide diversities evidenced in these regions.

The Japanese animals appear to be closely related to populations from the Russian Far East and are characterized by a very low level of genetic and nucleotide diversities. This result was already reported in other studies (Pavlenko, 1989, 1994a,b; Serizawa *et al.*, 2000, 2002). It may be explained by a recent colonization of Japan by a reduced number of colonizers probably originating from a single refuge located in Eastern Russia (Pavlenko, 1989, 1994a,b). This colonization would have been favoured by the appearance of land bridges between continental Asia and the Japanese archipelago during the last Quaternary cold phases (Chinzev & Machida, 2001). A similar phylogeographic pattern has been described for the coastal tiger beetle (Satoh *et al.*, 2004), termites (Park *et al.*, 2006), the red fox (Tsuda *et al.*, 1997), the sable (*Martes zibellina*) (Hosoda *et al.*, 1999), and the flying squirrel (*Pteromys volans*) (Oshida *et al.*, 2005).

Finally, the important genetic homogeneity between populations from the Russian Far East, southern Siberia, and central Asia strongly suggests that *A. peninsulae* may have colonized southern Siberia in the same way as *A. agrarius* (i.e. via Far eastern Russia). This colonization pattern could be explained by the same factors as for those of *A. agrarius* and, particularly, the important biogeographic barriers isolating China in the west and in the north. The isolation of Chinese populations of *A. peninsulae* was already suggested by Serizawa *et al.* (2002) who proposed two subspecific levels for this species in the mainland part of East Asia: *A. P. peninsulae* in Korea, Manchuria and eastern Siberia and *A. P. sowerbyi* in central China.

CONCLUSIONS

The present study demonstrated the existence of an important geographic structure of the genetic variability within each of the studied *Apodemus* species. This genetic differentiation could be linked not only to the cold and dry climates that characterized the Quaternary period in the Asian Far East, but also to the particular complex geology and topography of China and the Russian Far East.

In light of these results, China and, more generally, the Far East regions appear to be an important centre of diversification for the *Apodemus* species and a hot spot of biodiversity for this group of rodents, and probably for several hundreds of mammal species that occur there (Myers *et al.*, 2000; Wu *et al.*, 2000; Yu *et al.*, 2000; CI, 2007).

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