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Genetic structure of Pallas's squirrel (*Callosciurus erythraeus*) populations from artificial forests in Hongya County, Sichuan, China

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ABSTRACT

To understand levels of population differentiation in Pallas's squirrel (*Callosciurus erythraeus*) in fragmented habitats, we collected 83 samples from three patches of artificial forest in Hongya County, Sichuan Province, China. Sample numbers from each patch were as follows: 16 from Hanwang (HW), 27 from Muchansi (MCS) and 40 from Yanyandong (YYD). The mitochondrial DNA control region was sequenced and 18 haplotypes were observed. Our results showed that haplotype diversities of the three *C. erythraeus* populations were similar (0.771, 0.791 and 0.733). Fixation indices (F_{st}) of pairwise populations were between 0.21 and 0.31, and the estimated gene flow (*Nm*) was between 1 and 2. Analysis of molecular variance (AMOVA) showed that most molecular variation occurred within populations (74.82%); variances among populations were small but there was significant genetic differentiation. In addition, the neighbour-joining (NJ) tree showed three clades in the phylogenetic tree for population genetic structure. This was confirmed by the median-joining haplotype network. Furthermore, analysis of isolation by distance (IBD) showed that genetic differentiation among the three populations was positively related to geographical distance. However, tests of neutrality and the observed mismatch distribution of pairwise differences between sequences indicated that *C. erythraeus* populations were relatively stable in the past.

1. Introduction

Pallas's squirrel (*Callosciurus erythraeus*) is a small arboreal rodent widely distributed in China [1,2]. To promote local economic development, many secondary forests have been replaced by artificial ones. Along with these changes in forest, there have been reports of Pallas's squirrel causing damage to the artificial growth, beginning in the 1950s [3]. With more artificial forests being cultivated, squirrel damage has also become more serious since the 1980s [4–7].

Many studies have been done on the ecology, biology, and taxonomy of the species [1,2,8,9], but little attention has been given to the population history and population differentiation of the squirrel. However, intensive agricultural activities, the extensive road network and urbanization have cut the artificial forests into small, isolated patches. The consequences of habitat patches in a landscape are often negative such as habitat loss, genetic erosion, reduction of population sizes, and through random drift, increased levels of inbreeding and reduced gene flow on the natural populations [10–15]. Tests of neutrality and the observed mismatch distribution of pairwise differences between sequences may help to explore the population history [16,17].

The vertebrate mitochondria exhibits several peculiar features, such as maternal inheritance, the presence of single-copy orthologous genes, a lack of recombination, and a high mutation rate [18,19]. In particular, the mtDNA control region (D-loop) is not only a noncoding segment, but also the most variable part of the mtDNA [20,21]. So, as the marked molecule, the mtDNA control region is often used in population genetics and evolution [22,23].

Pallas's squirrels not only have high jump abilities among trees [24], but high dispersal abilities as well [25]. Did fragmentation of artificial forests affect gene flow and lead to genetic differentiation among Pallas's squirrel populations? What was the population history of the squirrel? To understand the levels of population differentiation and the population history of the squirrel in fragmented habitats, we employed mtDNA control region sequences to resolve the aforesaid questions.

2. Materials and methods

2.1. Sampling

With the help of the Forestry Bureau of Hongya, Sichuan Province, China, we legally captured 83 individuals of Pallas's squirrel

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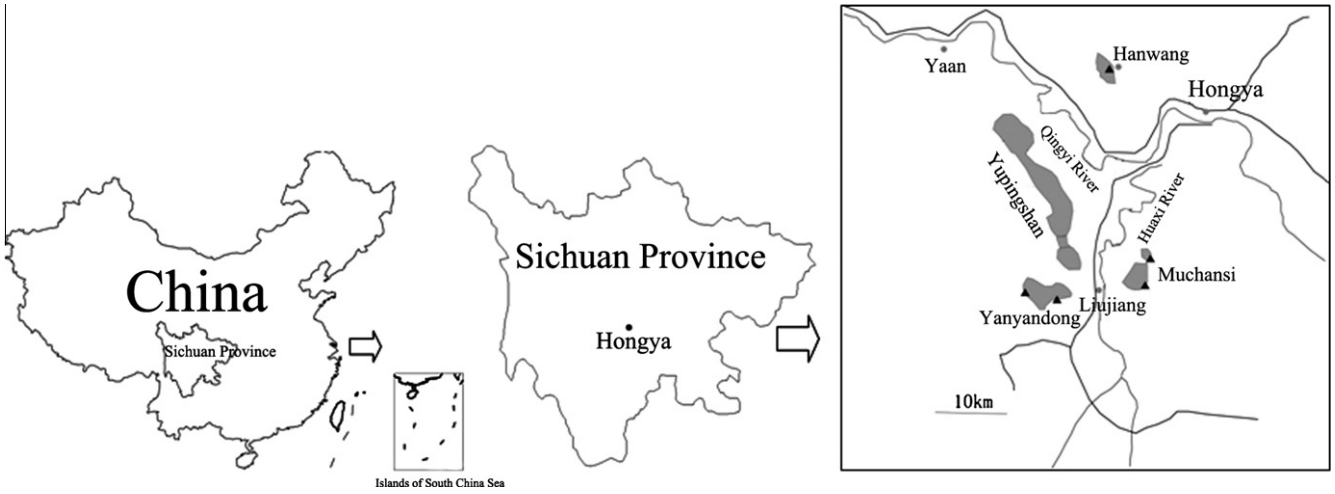

Fig. 1. Sampling localities of Pallas's squirrel.

from three different forest patches (Fig. 1) (Yupingshan was designed for ecological experiments. In order to avoid disturbance to the experiments, we did not catch any samples from this patch). Sample numbers from each patch were as follows: 16 from Hanwang (HW, 29°58′N, 103°14′E), 26 from Muchansi (MCS, 29°46′N, 103°16′E) and 41 Yanyandong (YYD, 29°42′N, 103°7′E). After morphological measurements, we removed the leg muscles from each specimen and immediately preserved them in absolute ethanol.

2.2. DNA extraction

Genomic DNA was extracted using a standard phenol–chloroform procedure [26] with minor modification. The extracted DNA were visualized on 1% agarose gels stained with ethidium bromide (EB) and then stored at −20 °C.

2.3. PCR and sequencing

The complete mtDNA control region was amplified using the primer pair L15933: 5′-CTCTGGTCTTGTAAACCAAAAATG-3′ (forward) and H637: 5′-AGGACCAAACCTTTGTGTTTATG-3′ (reverse) [27]. Polymerase chain reaction (PCR) amplification was carried out using DNA thermal cyclers (Eppendorf) with 25 μl of the reaction mixture containing 0.25 mM dNTP, 0.4 mM of each primer, 50 mM KCl, 10 mM Tris–HCl(pH 8.3), 1.5 mM $MgCl_2$, 1.25 units Taq DNA polymerase (Tiangen Biotech 2× Taq PCR MasterMix), and 200 ng of DNA template. The PCR protocol was an initial 4 min denaturation at 94 °C followed by 35 cycles of 40 s at 94 °C, 50 s at 51 °C, 1 min at 72 °C, and a final extension for 10 min at 72 °C before cooling to 22 °C. PCR products were separated by electrophoresis in a 1% agarose gel in 1× TAE buffer and stained with ethidium bromide (EB). For each sample, after electrophoresis, a band was observed and then excised from the gel. DNA was purified from the agarose with a DNA purification kit (Tiangen, Beijing) following the manufacturer's instructions. The purified mtDNA fragments were directly sequenced using an ABI3730XL automatic sequencer by the Invitrogen Biotechnology Limited Company (Shanghai, China).

2.4. DNA sequence alignment and analysis

All nucleotide sequences were aligned by Clustal X Version 1.83 and further refined manually [28]. We excluded gaps resulting from the alignment for genetic analysis. We detected nucleotide components and nucleotide polymorphic sites in the mtDNA control region by the MEGA Version 3.1 program [29]. Haplotype diversity (h) and nucleotide diversity (π) [30] were determined by DnaSP 4.0 [31]. To determine the population genetic structure within and among populations, we performed an analysis of molecular variance (AMOVA) using the Arlequin Version 3.0 software package [32]. Fixation indices [33] were calculated to assess overall genetic divergence and between paired populations. Estimates of gene flow (Nm) were derived using the equation: $N\mathrm{m} = [(1/F_{st}) - 1]/2$ [34,35].

We performed linear regression of estimates of $F_{st}/(1 - F_{st})$ between all pairs of populations against the logarithms of interpopulation geographical distances [34,35] and examined the result by the Mantel test using Isolation By Distance (IBD) software (www.bio.sdsu.edu/pub/andy/IBD.htm). We constructed a neighbour-joining tree [36] based on haplotypes according to Kimura's 2-parameter model [37], with *Callosciurus finlaysonii* (DDBJ accession number: AB181242) as an outgroup. In addition, we constructed a median-joining haplotype network [38] using Network 4.2.0.1 (http://www.fluxus-engineering.com) to determine the spatial distribution of haplotypes. To detect the historical demography of the squirrel population, we examined the observed mismatch distribution [39] of pairwise differences between sequences using the Arlequin Version 3.0 software package [32] and DnaSP 4.0 [31]. To test whether the sequences conformed to the expectations of selective neutrality, we used Tajima's D [40] and Fu's F_s [41] as the test of neutrality which is used to infer the population history. Populations that have passed through recent demographic expansions and genetic bottlenecks are usually predicted to have a unimodal wave in samples, whereas a bimodal or multimodal distribution of sharp peaks are often found in populations that have been constant over time [42,43].

3. Results

3.1. Molecular characteristics of the squirrel mitochondrial control region

We obtained the entire sequence (1079–1080 bp) of the mtDNA control region from 83 individuals and we adopted 1078 bp sequences of the D-loop to analyze population genetic variation in the squirrel after alignment. The overall base composition of the 83 sequences was as follows: T 32.4%, C 25.5%, A 30.6%, G 11.6%. Obviously, the D-loop of the squirrel is A/T rich, and this result is consistent with previous findings from other vertebrates. Sequence

Table 1
Haplotype diversity (h) and nucleotide diversity (π) of Pallas's squirrel based on mtDNA control region haplotype frequencies.

Haplotype		YYD population	MCS population	HW population
	11112222222222222222333799	n = 41	n = 26	n = 16
	68890188333444566788888567949	h = 0.771	h = 0.791	h = 0.733
	71873101567012448703567910920 6	π = 0.523%	π = 0.413%	π = 0.235%
Hap_1	ATCACCATCTTAGGGAGTCCTCCTCCTGCT	8		2
Hap_2	.....T..T.....C.....	11		
Hap_3	.....C.....A..CT.C....CA.C	15	2	
Hap_4	.....C.....A..CT.C...T.CA.C	1		
Hap_5	..GGG...TCG.A....C...T..T.....	1		
Hap_6	..GGG..C.....A.ACT.C....CA.C	1		
Hap_7	.....T.....			7
Hap_8	G.....T.....C.....			5
Hap_9	..G.....T.....			1
Hap_10	.....G...G...G.....T....			1
Hap_11	.....TC..A....C...T..T.....	1	8	
Hap_12	.....T.....C.....T.....	1	9	
Hap_13	.....T.....A..CT...TC...C..C		1	
Hap_14	.....T....A...C.....T....		1	
Hap_15	.C.....T.....C.....		3	
Hap_16	.....T.....A..CT...T...C..C		1	
Hap_17	.....T.....C.....	1	1	
Hap_18	.....C.....A..CT.C....CATC	1		

Table 2
Analysis of molecular variance (AMOVA) in populations of Pallas's squirrel populations.

Source of variation	Variance components	Percentage of variation	Fixation index	p-value
Among populations	0.788	25.18	0.253	0.0000
Within populations	2.343	74.82		

analysis of 1078 bp of the mitochondrial control region revealed 30 polymorphic sites, consisting of eight singleton variable sites and 22 parsimony informative sites.

3.2. Population genetic structure

Total nucleotide diversity is 0.00527 and haplotype diversity was 0.897. The haplotype diversities of the YYD, MCS and HW populations were similar (Table 1). Fixation indices (F_{st}) of pairwise populations were 0.21–0.31 and showed significant divergence between any two populations (Table 3). Analysis of molecular variance (AMOVA) showed that most molecular variation occurred within populations (74.82%), and variances among populations was small (25.18%) but there was significant genetic differentiation (Table 2). Analysis of genetic distance [$F_{st}/(1 - F_{st})$] and logarithm of geographic distance yielded a positive correlation, and the Mantel test also supported this result by significant r^2 values ($r^2 = 0.989$, $p < 0.001$).

Eighteen haplotypes were defined from the 83 sequences based on nucleotide diversity (Table 1) (GenBank accession numbers: GU474432–GU474449). One of the three populations had a unique haplotype and there were no common haplotypes among the three populations. The neighbour-joining tree (Fig. 2) showed that all haplotypes could be divided three groups. Clade I contained 14 haplotypes, among these, nine are from YYD, six from MCS and two from HW. Clade II contained three haplotypes, one was shared by YYD and MCS and the other two came from HW. Clade III contained only one haplotype and it came from HW. Obviously, compared with clade I, haplotypes in clade II and clade III all came from HW except for haplotype 1, suggesting that haplotypes from different populations were geographically distributed. These indicated that there was an obvious population genetic structure in the squirrels of the artificial forest. As shown in Fig. 3, the result of the median-joining haplotype network was consistent with that of the NJ tree.

Table 3
Values for the fixation index (below diagonal), associated P-values generated through a permutation test with 10,000 iterations (in parenthesis) and gene flow estimates (migrants per generation) (above diagonal) among different populations of Pallas's squirrel.

Population	YYD population	MCS population	HW population
YYD population		1.8598	1.3033
MCS population	0.21188(0.0002)		1.1398
HW population	0.27726(0.0004)	0.304913(0.0000)	

3.3. Historical demography

The selective neutrality test did not support the hypothesis that Pallas's squirrel had passed through a population bottleneck or expansion. No statistical significance for Tajima's D or Fu's F_s was observed for each population or for the entire sample set (Table 4). In addition, the shapes of the mismatch distributions of the three populations were all ragged and multimodal or bimodal (Fig. 4), suggesting the squirrel populations were relatively stable in the past.

4. Discussion

4.1. Genetic population structure

Human activities such as deforestation, road-building and agriculture have exacerbated the fragmentation of the landscape [44,45]. Habitat fragmentation has negative effects such as reduced gene flow, through random drift and exacerbated genetic differentiation in many wildlife species [10–15]. Pallas's squirrel is an arboreal rodent and it strongly depends upon forest habitat for survival. Human activities such as road-building and agriculture accelerated forest succession and forest fragmentation. Isolated and unforested habitat between forest patches could act as a

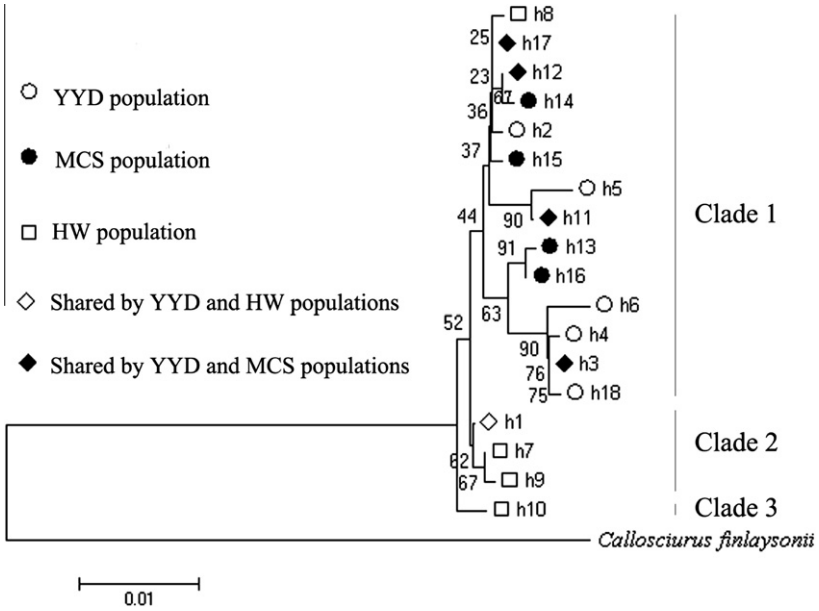

Fig. 2. Neighbour-joining tree based on haplotypes of Pallas's squirrel.

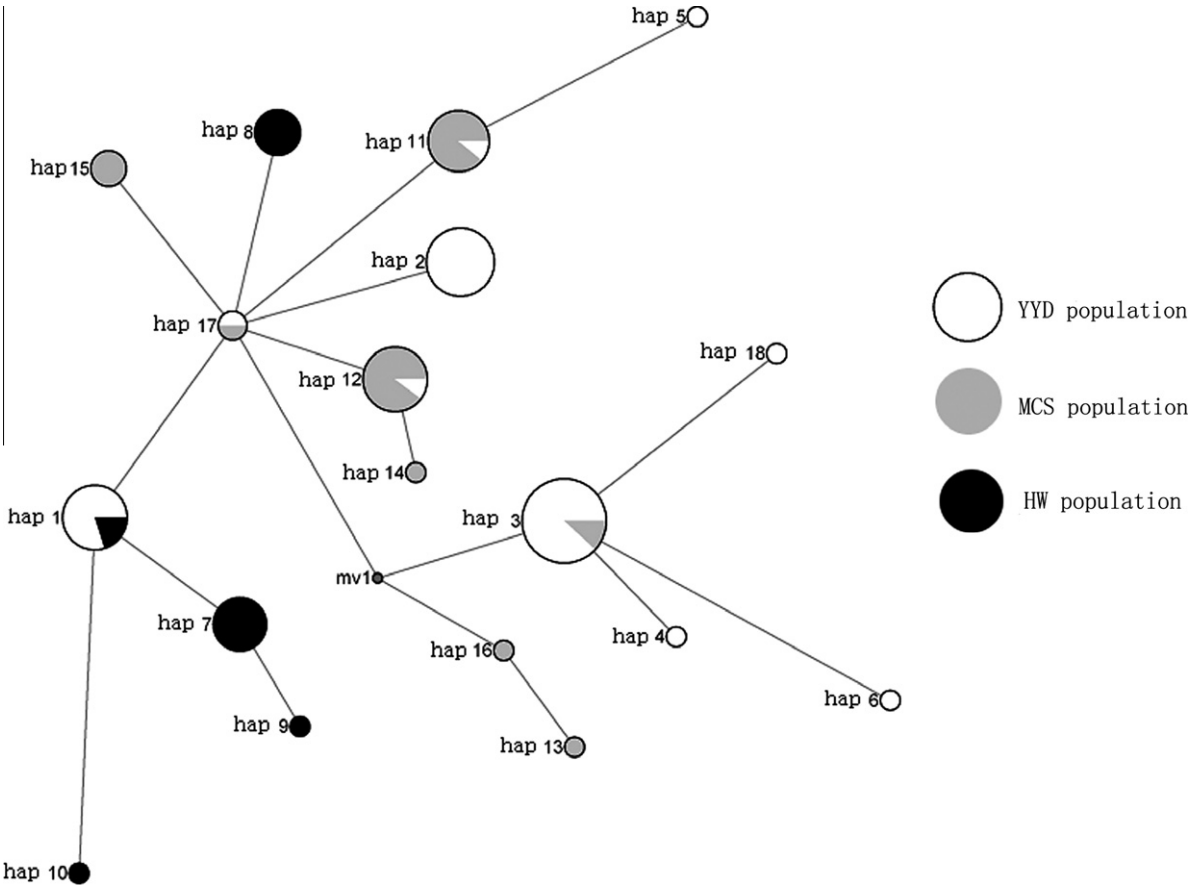

Fig. 3. Median-joining network of mtDNA haplotypes. The size of each disc and sector indicates the relative frequency of the corresponding haplotype in the entire data set.

barrier effect on the genetic interaction between squirrel populations. Fixation indices (F_{st}) are often used to determine the degree of genetic differentiation between organism populations, the greater the value of F_{st}, the greater the degree of genetic differentiation between populations [46]. *N*m may indicate the degree of gene flow between populations, if *N*m > 4, it indicates that the populations were panmictic [47], and if *N*m < 1, it means that there would be isolation between populations [48]. As Table 3 shows, pairwise F_{st} showed significance divergence between any two squirrel populations and gene flow (*N*m) estimates among

Table 4
Statistical tests of neutrality for Pallas's squirrel populations, associated *P*-value (in parenthesis).

	YYD population	MCS population	HW population	The entire sample set
Tajima' *D*	0.4931(0.736)	−0.0021(0.576)	−0.2423(0.420)	−0.1734(0.428)
F_s	1.8544(0.813)	1.1153(0.719)	0.9434(0.709)	−0.7478(0.488)

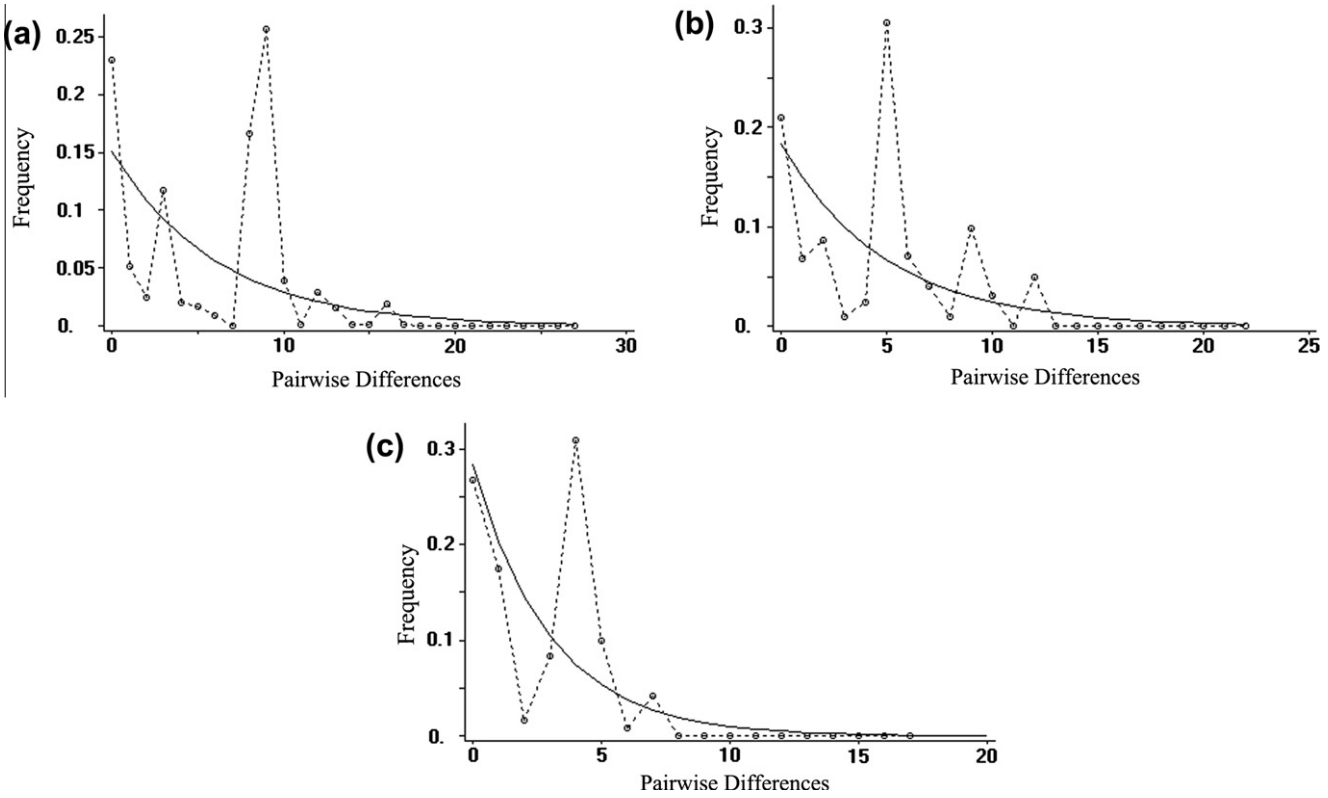

Fig. 4. Mismatch distribution for each population of Pallas's squirrel. (a–c) represent mismatch distributions for the Yanyandong, Muchansi and Hanwang populations, respectively. Dashed lines represent the observed distributions and solid lines represent the expected ones.

populations showed a low number of migrants per generation. In addition, the results of the AMOVA (Table 2) showed that most molecular variation occurred within the populations, and variances among populations were small but there was significant genetic differentiation. This situation indicated that patchy forest habitats reduced gene flow among populations and led to genetic differentiation.

Both the NJ tree (Fig. 2) and the Median-joining network indicated that haplotypes from different populations were geographically distributed. Geographically, the physical distances between YYD and HW and MCS and HW are approximately 32 km and 28 km, while the physical distance between YYD and MCS is 15 km. Haplotypes from the HW population clustered into distinct clades, suggesting that genetic differentiation among the squirrel populations was related to physical distance. What's more, the result from the analysis of IBD demonstrated that genetic distance $[F_{st}/(1-F_{st})]$ and the logarithm of geographic distance yielded a positive correlation (Mantel test, $r^2 = 0.989$, $p < 0.001$). Wright's theory of isolated distance [49] said that genetic differentiation was enhanced by physical distance among populations. His theory has been proved by many studies [50–52]. Pallas's squirrel is restricted in forest trees and has finished multiform behaviours such as foraging, mating, and springing by trees. So isolated and unforested habitat between forest patches no doubt has a barrier effect on gene flow, and further led to population differentiation.

4.2. Historical demography

The selective neutrality test and the mismatch distribution were often used to detect population history [16,17,41,42,53,54]. In our study, both nonsignificant Tajima's *D* or Fu'F_s and the pattern of multimodal peaks of the mismatch distribution indicated that the squirrel populations have been under demographic equilibrium. The forest ecosystem is a complex and stable terrestrial ecosystem. Complex food webs and relatively stable habitats could maintain animal populations in a stable state. For arboreal rodents, forest fragmentation not only led to reduced gene flow, increased population differentiation, but also population decline [55,56]. After the 1950s, human activities such as forestry practices, farming and road-building have dramatically changed the forest structure and exacerbated fragmentation of the artificial forest. As a result, habitat patches effected the pattern of genetic variation of the Pallas's squirrel populations. However, forest fragmentation had no effect on the population's historical demography. This situation may be related to the squirrel's adaptability for artificial forests. The squirrel mainly fed on plant material [1] and could change its food items as food resources supplied by the environment had dramatically changed [19]. Additionally, compared with natural or secondary forests, artificial forests are charactered by monoculture and a simple forest structure; furthermore, it is much disturbed by human activities. This situation was bad for the predators, so Pallas's squirrel had a good opportunity for

population growth. On the other hand, for the squirrel in the artificial forest, food shortages could be the restrictive factor for population growth [57,58]. Cai et al. [57] reported that the squirrel altered its food items by seasonal changes in food availability. Food resources were rich in summer and autumn, but scant in spring and winter. The fact that this squirrel stripped much bark is related to supplying enough nutrition and energy for the squirrel during the reproductive period in spring [58], and that this squirrel stripped much bark could be related to a shortage of food in winter [57]. Stripping bark could be the adaptive selection to the monoculture of the artificial forest. This adaptability for artificial forests could be the foundation of survival, reproduction and stability of the squirrel population.

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References

[1] W.J. Huang, Y.X. Chen, Y.X. Wen, Chinese Rodents, Fudan University Press, Shanghai, 1995.
[2] Y.X. Wang, A Complete Checklist of Mammal Species and Subspecies in China: A Taxonomic and Geographic Reference, China Forestry Publishing House, Beijing, 2003.
[3] B.Z. Guo, A preliminary survey of squirrel damage to forest trees in Taiwan, Technical Bulletin Experimental Forest 12 (1957) 1–20.
[4] B.Z. Guo, W.Z. Lin, R.K. Fang, Observation on squirrel's damage to plantation of Taiwan and Chinese Guger Tree-with special reference to squirrel problems in the Huisun Experimental Forest, National Zhongxing University, Quarterly Journal of Chinese Forestry 17 (3) (1984) 15–29.
[5] J.H. Ran, Q. Lin, S.Y. Liu, et al., A study on method of prevention and cure against rodent pests in artificial forestry in Sichuan, Journal of Sichuan Forestry Science and Technology 20 (3) (1999) 41–43.
[6] S.Y. Liu, J.H. Rang, Q. Lin, et al., The reason analysis of rodent damage in plantation, Forest Research 15 (5) (2002) 614–619.
[7] W. Xu, J.H. Ran, Studies and control of *Callosciurus erythraeus*, Journal of Sichuan Forestry Science and Technology 25 (4) (2004) 16–21.
[8] Z.D. Wang, B.Z. Guo, Squirrels are harmful to economical forest of Taiwan, Monthly of Science Development 8 (6) (1980) 527–550.
[9] Y.S. Lin, L.L. Li, The behavioral study of the red-bellied tree squirrel (*Callosciurus erythraeus*), Biology Science 25 (1985) 3–12.
[10] S. Lampila, L. Kvist, R. Wistbacka, et al., Genetic diversity and population differentiation in the endangered Siberian flying squirrel (*Pteromys volans*) in a fragmented landscape, European Journal of Wildlife Research 55 (4) (2009) 397–406.
[11] A. Hille, K. Liebal, B. Mosch, H. Pellmann, et al., An RAPD (random amplified polymorphic DNA) analysis of genetic population structure of *Balea biplicata* (Gastropoda: Clausiliidae) in fragmented floodplain forests of the Elster/Saale riparian system, Biochemical Genetics 41 (2003) 175–199.
[12] A.V. Geert, F.V. Rossum, L. Triest, Genetic diversity in adult and seedling populations of *Primula vulgaris* in a fragmented agricultural landscape, Conservation Genetics 9 (2008) 845–853.
[13] A. Kolb, Reduced reproductive success and offspring survival in fragmented populations of the forest herb *Phyteuma spicatum*, Journal of Ecology 93 (2005) 1226–1237.
[14] D. Fulgione, V. Maselli, G. Pavarese, et al., Landscape fragmentation and habitat suitability in endangered Italian hare (*Lepus corsicanus*) and European hare (*Lepus europaeus*) populations, European Journal of Wildlife Research 55 (4) (2009) 385–396.
[15] A. Young, T. Boyle, T. Brown, The population genetic consequences of habitat fragmentation for plants, Trends in Ecology and Evolution 11 (1996) 413–418.
[16] K. Neumann, H. Jansman, A. Kayser, et al., Multiple bottlenecks in threatened western European populations of the common hamster *Cricetus cricetus* (L.), Conservation Genetics 5 (2004) 181–193.
[17] K.E. Galbreath, J.A. Cook, Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia, Molecular Ecology 13 (2004) 135–148.
[18] G. Pesole, C. Gissi, A.D. Chirico, et al., Nucleotide substitution rate of mammalian mitochondrial genomes, Journal of Molecular Evolution 48 (1999) 427–434.
[19] A. Larizza, G. Pesole, A. Reyes, et al., Lineage specificity of the evolutionary dynamics of the mtDNA D-loop region in rodents, Journal of Molecular Evolution 54 (2002) 145–155.
[20] R.L. Cann, W.M. Brown, A.C. Wilson, Polymorphic sites and the mechanism of evolution in human mitochondrial DNA, Genetics 106 (3) (1984) 479–499.
[21] P.W. Wenink, A.J. Baker, M.G.J. Tilanus, Mitochondrial control region sequences in two shorebird species: the turnstone and the dunlin, and their utility in population genetic studies, Molecular Biology and Evolution 11 (1994) 22–31.
[22] A.K.Z. Guillén, G.M. Barrett, O. Takenaka, Genetic diversity among African great apes based on mitochondrial DNA sequences, Biodiversity and Conservation 14 (2005) 2221–2233.
[23] T. Matsuhashi, R. Masuda, T. Mano, et al., Microevolution of the mitochondrial DNA control region in the Japanese brown bear (*Ursus arctos*) population, Molecular Biology and Evolution 16 (1999) 676–684.
[24] J.H. Ran, W. Xu, H.X. Cai, et al., A primary study on the biology of red-bellied squirrels, Journal of Sichuan Forestry Science and Technology 26 (6) (2005) 8–10.
[25] M. Setoguchi, Food habits of red-bellied tree squirrels on a small island in Japan, Journal of Mammalogy 71 (4) (1990) 570–578.
[26] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, second ed., Cold Spring Harbor Laboratory Press, New York, 1989.
[27] T. Oshida, K. Ikeda, K. Yamada, et al., Phylogeography of the Japanese giant flying squirrel, *Petaurista leucogenys*, based on mitochondrial DNA control region sequences, Zoological Science 18 (2001) 107–114.
[28] J.D. Thompson, T.J. Gibson, F. Plewniak, et al., The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Research 24 (1997) 4876–4882.
[29] S. Kumar, K. Tamura, M. Nei, MEGA 3.1 integrated software for molecular evolutionary genetics analysis and sequence alignment, Briefings in Bioinformatics 5 (2004) 150–163.
[30] M. Nei, Molecular Evolutionary Genetics, Columbia University Press, New York, 1987.
[31] J. Rozas, J.C. Sanchez-DelBarrio, X. Messeguer, et al., DnaSP, DNA polymorphism analysis by the coalescent and other methods, Bioinformatics 19 (2003) 2496–2497.
[32] L. Excoffier, G. Laval, S. Schneider, Arlequin Version 3.0: an integrated software package for population genetics data analysis, Evolutionary Bioinformatics Online 1 (2005) 47–50.
[33] L. Excoffier, P. Smouse, J. Quattro, Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data, Genetics 131 (1992) 479–491.
[34] B.S. Weir, C.C. Cockerham, Estimating *F*-statistics for the analysis of population structure, Evolution 38 (1984) 1358–1370.
[35] F. Rousset, Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance, Genetics 145 (1997) 1219–1228.
[36] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Molecular Biology and Evolution 4 (1987) 406–425.
[37] M. Kimura, A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences, Journal of Molecular Evolution 16 (1980) 111–120.
[38] H.J. Bandelt, P. Forster, A. Röhl, Median-joining networks for inferring intraspecific phylogenies, Molecular Biology and Evolution 16 (1999) 37–48.
[39] M. Slatkin, R.R. Hudson, Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations, Genetics 129 (1991) 555–562.
[40] F. Tajima, Statistical method for testing the neutral mutation hypothesis by DNA polymorphism, Genetics 123 (1989) 585–595.
[41] Y.X. Fu, Statistical tests of neutrality of mutations against population growth, Hitchhiking and Background Selection, Genetics 147 (1997) 915–925.
[42] A.R. Rogers, H. Harpending, Population growth makes waves in the distribution of pairwise genetic differences, Molecular Biology and Evolution 9 (1992) 552–569.
[43] S. Schneider, L. Excoffier, Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA, Genetics 152 (1999) 1079–1089.
[44] D.J. Bender, T.A. Contreras, L. Fahrig, Habitat loss and population decline: a meta-analysis of the patch size effect, Ecology 79 (1998) 517–533.
[45] S. Gurdebeke, J.P. Maelfait, T. Backeljau, Contrasting allozyme and RAPD variation in spider populations from patchy forest habitats, Genetica 119 (2003) 27–34.
[46] S. Wright, Evolution and the Genetics of Populations Variability within and among Natural Populations, University of Chicago Press, Chicago, 1978.
[47] D.L. Hartl, A.G. Clark, Principle of Population Genetics, second ed., Sinauer Associates Incorporated Publishers, Sunderland, 1989.
[48] J.E. Neigel, Is F_{ST} obsolete?, Conservation Genetics 3 (2002) 167–173
[49] S. Wright, Isolation by distance, Genetics 28 (1943) 114–138.
[50] I. Trizio, B. Crestanello, P. Galbusera, et al., Geographical distance and physical barriers shape the genetic structure of Eurasian red squirrels (*Sciurus vulgaris*) in the Italian Alps, Molecular Ecology 14 (2) (2005) 469–481.
[51] C.H. Floyd, D.H. Van Vuren, B. May, Marmots on great basin mountaintops: using genetics to test a biogeographic paradigm, Ecology 86 (8) (2005) 2145–2153.

[52] B. Goossens, L. Chikhi, P. Taberlet, et al., Microsatellite analysis of genetic variation within Alpine marmot populations in the French Alps, Molecular Ecology 10 (1) (2001) 41–52.
[53] T. Atarhouch, L. Rüber, E.G. Gonzalez, et al., Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*), Molecular Phylogenetics and Evolution 39 (2006) 373–383.
[54] S.J. Yang, Z.H. Yin, X.M. Ma, et al., Phylogeography of ground tit (*Pseudopodoces humilis*) based on mtDNA: evidence of past fragmentation on the Tibetan Plateau, Molecular Phylogenetics and Evolution 41 (2006) 257–265.
[55] S. Lampila, L. Kvist, R. Wistbacka, et al., Genetic diversity and population differentiation in the endangered Siberian flying squirrel (*Pteromys volans*) in a fragmented landscape, European Journal of Wildlife Research 55 (2009) 397–406.
[56] E.M. Barratt, J. Gurnell, G. Malarky, et al., Genetic structure of fragmented populations of red squirrel (*Sciurus vulgaris*) in the UK, Molecular Ecology 8 (1999) 855–863.
[57] H.X. Cai, J.H. Ran, J.P. Zhang, et al., Study on damage of *Callosciurus erythraeus* by seasonal change and its food habit, Journal of Sichuan Forestry Science and Technology 22 (3) (2001) 21–24.
[58] L. Dong, M. Ji, W. Xu, et al., Relation of damage and reproduction of red-bellied squirrels in plantation, Sichuan Journal of Zoology 28 (2) (2009) 197–201.