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ORIGINAL PAPER

Phylogeographic analysis of the fir species in southern China suggests complex origin and genetic admixture

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Abstract

• ***Context*** Fir species are mainly distributed across the high latitudes, preferring cold climates. The phylogeographic origins of those subtropical fir species that occur in low latitudes in southern China remain elusive, as does the nature of any inter-lineage hybridization.

• ***Aims*** In fir species, mitochondrial DNA (mtDNA) is maternally inherited and chloroplast DNA (cpDNA) is paternally inherited, and the genetic variations in the two make them particularly useful for examining species' hybridizations and evolutionary histories.

• ***Materials and methods*** We sequenced four DNA fragments: two of mtDNA and two of cpDNA for 161 individuals from four subtropical endangered fir species (*Abies beshanzuensis*, *Abies ziyuanensis*, *Abies yuanbaoshanensis*, and *Abies fanjingshanensis*) and two more northerly distributed ones (*Abies recurvata* and *Abies fargesii*) from central and southwest China.

• ***Results*** Three mitotypes and four chlorotypes were recovered from the four southern species. In most populations, individuals share a single fixed mitotype and chlorotype. Three mitotypes clustered into two distinct clades, each associated with one of the northern species examined. For four chlorotypes, one occurred in *A. ziyuanensis*, *A. beshanzuensis*, *A. fargesii*, and *A. recurvata*, another in *A. ziyuanensis* and *A. recurvata*, the remaining two differed from others by two mutations exclusively in *A. fanjingshanenis* and *A. yuanbaoshanensis.*

• ***Conclusion*** Phylogeographic origins of the subtropical fir species are complex, and genetic admixtures occurred during the evolutionary history of *A. ziyuanensis*. The genotyped populations further provide basic frames for genetic delimitation and effective conservation of these endangered species in the future.

Keywords Fir species · mtDNA · cpDNA · Phylogeography · Genetic admixture

1 Introduction

Past climatic oscillations greatly affected the distributions of most plants (Hewitt 2004), although the effects varied between localities and groups of species (Avise 2004). Temperate plants generally retreated to refugia at low altitudes or latitudes when the climate became cold and recolonized their former ranges as temperatures subsequently rose (Avise 2004). However, such range shifts may still have left populations in nunatak refugia (glacial refugia on mountain peaks that protruded above the glaciers in the core of a mountain range) at high altitudes or in high latitudes during cold periods, as well as in isolated mountains at low latitudes when the climate warmed once more (Holderegger and Thiel-Egenter 2009; Stehlik et al. 2002). Any such isolated populations probably had extremely low genetic diversity and high genetic

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Contribution of the co-authors J.L. designed research; J.L., B.T., and G.R. collected samples; Y.P., J.W., Q.G, and B.T. performed research; Y.P. and J.L. analyzed data; and Y.P., S.Y. and J.L. wrote the paper.
Yanling Peng and Shuming Yin contributed equally to the work.

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differentiation, and some may have finally evolved into new (cryptic or morphologically distinct) species due to the strong genetic drift caused by bottlenecks or founder effects (Avise 2004). In particular, lineages that had retreated or recolonized from different areas or refugia usually hybridized with one another as their ranges merged, producing numerous hybrid populations or species (e.g., Abbott et al. 2010).

Fir species usually occur in the Northern Hemisphere in high latitude regions or at high altitudes. The forest ecosystems dominated by fir trees provide a basic home for a great diversity of animals and plants (Farjon and Rushforth 1989; Florin 1963; Liu 1971). However, a limited number of fir species are found further south in subtropical mountains; such species occur in discrete, disconnected populations (Florin 1963; Liu 1971). In China, there are 22 fir species, most of which are found in high latitude or high altitude regions in northern, central, or southwest China (Fu et al. 1999). However, in southern China, there are four highly endangered species: *Abies beshanzuensis* M. H. Wu, *Abies ziyuanensis* L. K. Fu & S. L. Mo, *Abies yuanbaoshanensis* Y. J. Lu & L. K. Fu, and *Abies fanjingshanensis* W. L. Huang (Fu et al. 1999) (Fig. 1). These species have a very restricted distribution on the subtropical mountain tops, and the very small numbers of surviving individuals mean that they are extremely endangered (Farjon 1990; Liu 1971; Ning et al. 2005; Xiang 2001; Zhang et al. 2004). These four species have been classified under two infrageneric sections: *A. beshanzuensis* and *A. ziyuanensis* were placed in Sect. *Momi*, which is morphological similarity to the *Abies recurvata* complex that occurs in southwest China; *Abies fanjingshaensis* and *Abies yuanbaoshanensis* were placed in Sect. *Pseudopicea*, which is similar to the *Abies fargesii* complex from central China (Farjon and Rushforth 1989). Phylogenetic analyses based on internal transcribed spacer data suggested that these two sections clustered into monophyletic clades but with low bootstrap support (Xiang et al. 2009). It has often been suggested that these subtropical fir species originated from southward retreats of the fir forests of central or southwest China (Florin 1963; Li 1995; Liu 1971; Liu et al. 2002; Ying 1989). However, actual phylogeographic relationships between them and those species occurring further north remain unknown. Did they originate only from the fir forests of central China or from southwest China or from both areas? If the southward retreats of fir forests from two regions created these subtropical species, did introgressions or hybridization occur between them?

In order to answer these questions, we sequenced mitochondrial (mt) and chloroplast (cp) DNA fragments from four subtropical and two northern species from different regions. Like most other conifers, these cytoplasmic types of DNA in *Abies* are haploid; mtDNA is maternally

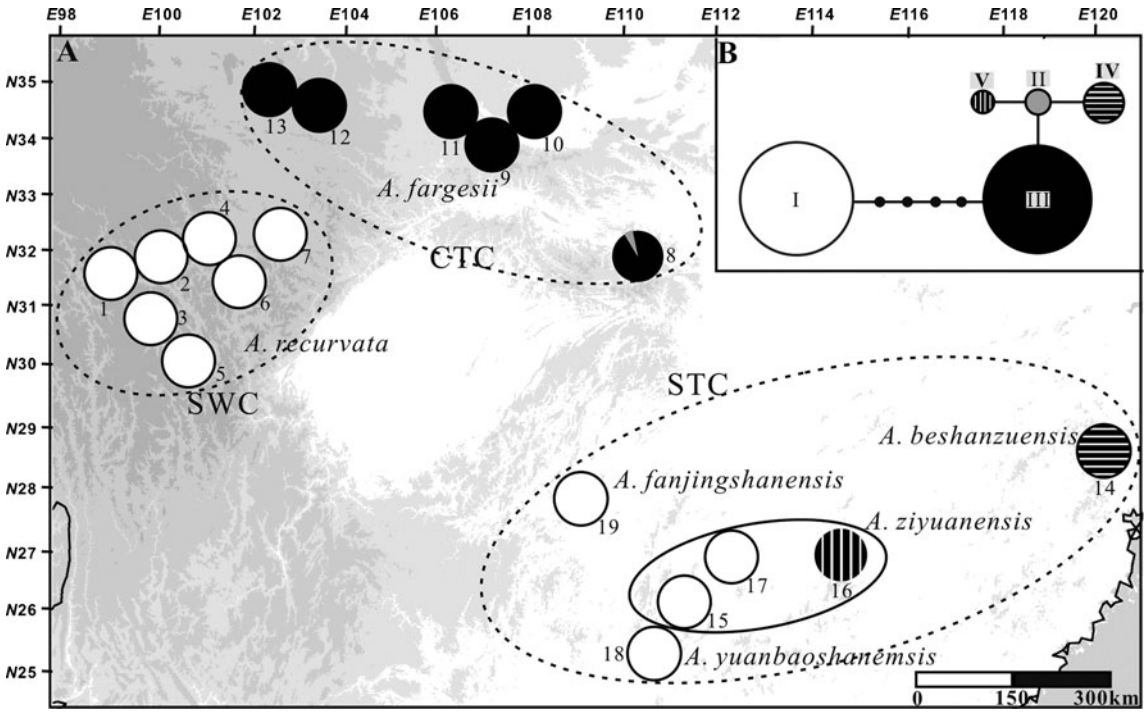

Fig. 1 Distributions (**a**) and networks (**b**) of mitotypes recorded in six closely related *Abies* spp. The range of each region is indicated by *dash line*. In the networks, each mitotype is represented by a *circle*, the size of which is proportional to its frequency over all populations

transmitted via seed while cpDNA is paternally transmitted via pollen (Liepelt et al. 2002). Thus, these DNA data together provide a sound basis for tracing range shifts (via seed) during retreats and recolonizations and for identifying potential hybrid origins of the current species or populations through maternal and paternal relationships (e.g., Liepelt et al. 2002, 2010; Song et al. 2003).

2 Materials and methods

2.1 Sampling design

Samples were obtained from 161 individuals of 19 populations spanning the geographic range of each of the six focal species (see Fig. 1 and Table S1). Of these samples, 38 individuals (19.3% in total) of six populations representing four southern species were sampled due to their limited distribution. For example, there are only three surviving individuals of *A. beshanzuensis* (Ning et al. 2005; Zhang et al. 2004); we sampled two adult individuals. More populations and individuals of *A. ziyuanensis* are known. We found and collected all three mature individuals at Yinzhulaoshan. The other two known populations contain more adult individuals (more than 20). In each population, therefore, we sampled seven mature individuals that were growing at least 20 m apart; the other individuals were growing very close to the sampled trees. Only one population of *A. yuanbaoshanensis* was found (Ning et al. 2005; Zhang et al. 2004), and we located fewer than 20 adult individuals; we collected material from seven individuals each about 10 m from the next. The only known population of *A. fanjingshanensis* is large and contains more than 50 mature individuals; we therefore sampled 12 adult individuals that were about 100 m apart. For the two more widely distributed, northerly species, we randomly chose six or seven populations from across their entire range. In principal, 10 individuals separated by at least 100 m were collected from each population. A palm Extrex GIS (Garmin) was used to measure the latitude, longitude, and altitude of each sampling site.

2.2 DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from a sample of approximately 20 mg of silica gel-dried needles, using the hexadecetytrmethyl ammonium bromide procedure (Doyle and Doyle 1987). Intron 4 of subunit 5 of the mitochondrial nicotinamide adenine dinucleotide dehydrogenase gene (*nad*5 intron 4) and Intron 1 of subunit 7 of the same gene (*nad*7 intron 1) were amplified and sequenced, using primers described by Wu et al. (1998) and Jaramillo-Correa et al. (2004), respectively. In addition, cpDNA *trn*S-G and *trn*L-F were amplified and sequenced following Demesure et al. (1995) and Taberlet et al. (1991), respectively. Polymerase chain reactions (PCRs) were performed in a 25-μL volume, which contained 10–40 ng plant DNA, 50 mM Tris–HCl, 1.5 mM $MgCl_2$, 250 μg/mL BSA, 0.5 mM dNTPs, 2 μM of each primer, and 0.75 U Taq polymerase. The PCR amplifications were conducted using the following temperature profile: 4 min at 94°C, followed by 37 cycles of 40 s at 94°C, 40 s of annealing at 58°C (for mtDNA) or 60°C (for cpDNA), and 1.2 min at 72°C, with a final 7 min extension at 72°C.

We then purified the PCR products using a TIANquick Midi Purification Kit (TIANGEN, Beijing, China). Sequencing reactions were performed with the PCR primers described above using an ABI PRISM BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). We aligned the sequences obtained with Clustal X (Thompson et al. 1997), following manual modifications in MEGA4 (Tamura et al. 2007). The newly recovered sequences have been deposited in the NCBI Genbank under Accession Numbers JN584218-JN584263. In addition, the accession numbers of *A. fargesii* sequences have been supported in a previous study (see Wang et al. 2011).

2.3 Phylogenetic and phylogeographic analyses

The limited numbers of mutations associated with mtDNA and cpDNA in firs preclude the construction of phylogenetic trees using the maximum parsimony and maximum likelihood methods. We therefore followed previous researchers (Liepelt et al. 2002, 2010; Jaramillo-Correa et al. 2004, 2008) and constructed minimum-spanning trees for the recovered haplotypes using the software Network version 4.2.0.1 (available at http://www.fluxus–engineering.com; Bandelt et al. 1999). For phylogeographic analyses, we defined three regions based on species distributions: southwest China (SWC), central China (CTC), and southern China (STC) (See Fig. 1 and Table S1). Population structure within and between regions was analyzed separately for mtDNA and cpDNA. The average gene diversity within populations (H_S), total gene diversity (H_T), and the coefficients of differentiation G_{ST} and N_{ST} were estimated for each species. G_{ST} is calculated solely on the basis of the frequencies of haplotypes, whereas N_{ST} takes into account the similarities (phylogenetic relatedness) between haplotypes; calculations were performed using PERMUT (Pons and Petit 1996; available at http://pierroton.inra.fr/labo/software/permutcpssr). Hierarchical partitioning of diversity among species, populations, and individuals was based on analyses of molecular variance (AMOVA), using the software Arlequin version 3.1 (Excoffier et al. 2006), with significance tests based on 1,000 permutations.

3 Results

3.1 mtDNA variation and distribution

Polymorphisms were recorded within both mtDNA regions examined. Three variants of *nad*7 intron 1 were the result of one indel, and four variants of *nad*5 intron 4 were recovered as a result of one indel and three nucleotide substitutions (Table S2). By considering the two polymorphic mtDNA regions together, we identified five distinct haplotypes (I–V), clustering into two distinct clades in the minimum-spanning tree, separated by five mutations (Fig. 1). Mitotype I, which represented one distinct clade, was found widely across four species: *A. yuanbaoshanensis*, *A. fanjingshanensis*, *A. ziyuanensis*, and *A. recurvata*. However, four remaining mitotypes belonging to the other clade were found in *A. beshanzuensis* (IV), *A. ziyuanensis* (V), and *A. fargesii* (II and III), respectively (Fig. 1).

Low levels of genetic variation were detected within the fir species studied herein using two mitochondrial fragments. No polymorphism was identified within *A. recurvata*, *A. yuanbaoshanensis*, *A. fanjingshanensis*, or *A. beshanzuensis* (Fig. 1 and Table S1). However, all populations of *A. ziyuanensis* are monomorphic with two different mitotypes fixed.

3.2 cpDNA variation and distribution

One indel and 13 substitutions were found in two cpDNA sequences, which combined into 17 chlorotypes (A–Q) (Fig. 2 and Table S2). These chlorotypes can be tentatively divided into three clades. The first comprises those chlorotypes recovered from *A. fargesii* (A–H), one of which (A) was also present in the southern species. The second includes those chlorotypes from *A. recurvata*, one of which (I) was also present in one southern species. The third clade comprises the two chlorotypes (L and M) that are found exclusively in the southern species. This clade obviously originated from the second clade belonging to *A. recurvata* because L is closely related to I, being separated by only one step (Fig. 2). The two widely distributed chlorotypes in the former two clades (A and I) are separated by only one step. Only four of 17 chlorotypes were found in the four southern species. Chlorotype A was present in *A. beshanzuensis* and two populations of *A. ziyuanensis* (Fig. 2). This chlorotype is closely related to others occurring exclusively in *A. fargesii* (for example, chlorotype B). Another chlorotype I was found to have a discontinuous geographical distribution; it was present in one population of *A. ziyuanensis* as well as in *A. recurvata* in southwest China. The final two chlorotypes (L and M) found in the southern China were restricted to *A. yuanbaoshanensis* and *A. fanjingshanensis*. A total of 14 chlorotypes were found in the two widespread northern species, *A. fargesii* and *A. recurvata*.

The two widespread northern species (*A. fargesii* and *A. recurvata*) exhibit high diversity and have numerous private chlorotypes, while all the southern species exhibit extremely low polymorphism (Table S1). In addition, as with the mtDNA, genetic variation within both populations and species is extremely low. However, genetic variation between the populations of *A. ziyuanensis* is high because each is associated with a different chlorotype (Fig. 2 and Table 1).

4 Discussion

Our population genetic analyses of the four fir species in southern China based on mtDNA and cpDNA variations suggested that these species have complex phylogeographic origins. Especially, we found distinct genetic admixtures possibly due to hybridization and introgression in one of them (*A. ziyuanensis*). In addition, range expansions based on the genetic data are distinct in two northern species, indicating that they might have retreated southward and recolonized northward in response to the past climatic changes. The southward retreats of the northerly distributed species probably gave rise to the production of these subtropical fir species. The genotyped population data further provide basic frames for genetic delimitation and effective conservation of these endangered subtropical fir species in the future.

4.1 Phylogeographic history

Our results suggested that two major mitotypes, one from each of two clades, characterized the two widespread and northerly fir species (Fig. 1). However, the mitotypes recovered from *A. beshanzuensis* (IV) and one population of *A. ziyuanensis* (V) belonged to the same clade as those (II and III) recovered from *A. fargesii* while *A. yuanbaoshanensis*, *A. fanjingshanensis*, and two populations of *A. ziyuanensis* shared a mitotype (I) with the northern species *A. recurvata.* Given that the two clades differed by four mutations, the fragmentation of the ancestral genetic pool cannot have occurred very recently although we could not exclude it totally. If we assume that these two clades formed before the colonization of southern China (Florin 1963; Li 1995; Liu 1971; Liu et al. 2002; Ying 1989), the phylogenetic relationships between mitotypes indicate that the fir species in southern China possibly originated from two different more northerly regions, namely central and southwest China. In fact, the chlorotype networks seem to support this possibility. Chlorotype A is associated with two species, *A. beshanzuensis* and *A. ziyuanensis*, and it is also found widely in *A. fargesii* in central China. Similarly, chlorotype I was found in *A. ziyuanensis* and *A. recurvata*, which is present in southwest China. However, two chlorotypes found in *A.*

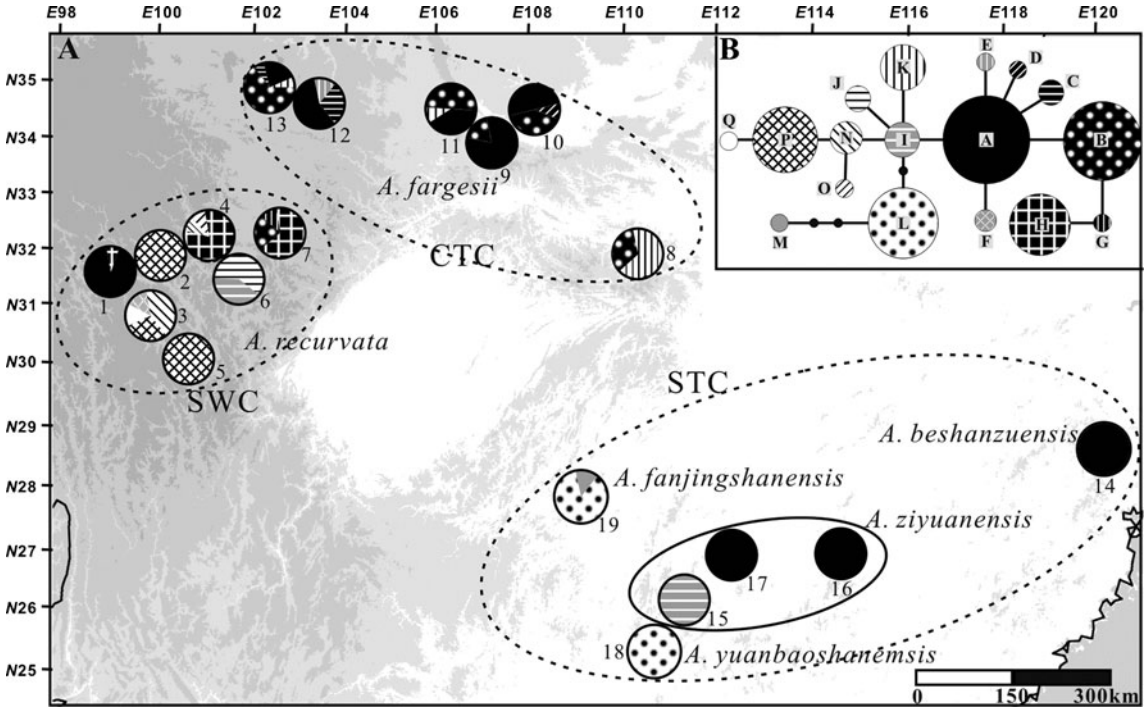

Fig. 2 Distributions (**a**) and networks (**b**) of chlorotypes recorded in six closely related *Abies* spp. The range of each region is indicated by *dash line*. In the networks, each chlorotype is represented by a *circle*, the size of which is proportional to its frequency over all populations

yuanbaoshanensis and *A. fanjingshanensis* did not appear in the two northerly species. These two chlorotypes comprised a separate clade in the network, and they are most closely related to chlorotype I. These two chlorotypes probably originated independently in southern China from an ancestral chlorotype resembling I. In addition, it should be noted that

Table 1 AMOVA for mtDNA and cpDNA variation in six closely related *Abies* species

Regions	Source of variation	*d.f.*	SS	VC	VA (%)	Fixation index
Mitotypes						
Total	Among groups	2	137.6	1.270	84.57	F_{CT}=0.846*
	Among populations within groups	16	29.83	0.225	15.01	F_{SC}=0.973*
	Within populations	142	0.889	0.006	0.42	F_{ST}=0.996*
	Total	160	168.3	1.502		
SWC	Among populations	6	0.000	0.000	0.00	F_{ST}=0.000*
	Within populations	52	0.000	0.000	0.00	
CTC	Among populations	5	0.095	0.000	2.28	F_{ST}=0.023
	Within populations	58	0.889	0.015	97.72	
STC	Among population	3	29.74	0.991	100.0	F_{ST}=1.000*
	Within population	32	0.000	0.000	0.000	
Chlorotypes						
Total	Among groups	2	48.34	0.351	29.37	F_{CT}=0.294*
	Among populations within groups	16	79.62	0.570	47.67	F_{SC}=0.675*
	Within populations	142	38.99	0.275	22.96	F_{ST}=0.770*
	Total	160	166.9	1.196		
SWC	Among populations	6	43.05	0.849	79.1	F_{ST}=0.791*
	Within populations	52	11.66	0.224	20.9	
CTC	Among populations	5	8.794	0.129	8.14	F_{ST}=0.235*
	Within populations	58	24.79	0.424	76.54	
STC	Among populations	5	27.59	0.905	91.33	F_{ST}=0.913*
	Within populations	32	2.750	0.086	8.63	

d.f. degrees of freedom, *SS* sum of squares, *VC* variance component

**P<0.001; 1,000 permutations

more haplotypes at both mtDNA and cpDNA might have existed but vanished with the range shrinkage of these southern fir species. Therefore, our analyses suggested these southern fir species have complex phylogeographic origins, probably from southern retreats of the northern species, occurring in CTC, SWC, or other northerly distributed regions.

For the two northerly species, our population genetic analyses of both mtDNA and cpDNA data suggested that they had experienced extensive range expansions in the recent past. All populations of each species were dominated by a single mitotype (Fig. 1). This is consistent with numerous reports that range expansion from local or remote refugia promoted the widespread fixing of a single haplotype for mtDNA in conifers, which is associated with few mutations (Avise 2004; Du et al. 2009). However, a range expansion could have also resulted in a dominant haplotype with multiple rare alleles (a star-phylogeny pattern) for DNA fragments with a fast mutation rate (for example, cpDNA in conifers) (Avise 2004). In both *A. recurvata* and *A. fargesii*, we found numerous rare chlorotypes, possibly supporting an expansion hypothesis, although there was no distinct star-phylogeny pattern. The actual mutation rates of mtDNA and cpDNA in the *Abies* genus are not known. It is also not yet known when the fir species from the central and southwest China colonized southwards as a result of decreasing temperature and when subsequent expansions occurred as the climate warmed. However, all these genetic data seem to be consistent with previous speculations that the southward retreats of the northerly distributed species during the cold climate age gave rise to the production of the subtropical fir species in southern China (Florin 1963; Li 1995; Liu 1971; Liu et al. 2002; Ying 1989).

4.2 Genetic delimitation and hybridization

Our genetic analyses suggested that a combination of the recovered mitotypes and chlorotypes could be used to characterize three of the four rare species that we examined; the exception was *A. ziyuanensis*. Mitotype IV was only found in *A. beshanzuensis*, although this species shared chlorotype A with *A. ziyuanensis* and *A. fargesii*. Although *A. yuanbaoshanensis* and *A. fanjingshanensis* shared a mitotype (I) with *A. ziyuanensis* and *A. recurvata*, chlorotype L was fixed in both of them, while chlorotype M was only found in *A. fanjingshanensis*. Although these two species can be distinguished from the other fir species and from each other by the presence of a single species-specific chlorotype (M) (Du et al. 2009; Tsumura and Suyama 1998), taxonomic and genetic delimitations between them need further studies based on more data.

As noted before, the networks of both mitotypes and chlorotypes suggested that these three species probably originated from different southward movements of fir species: *A. beshanzuensis* from the *A. fargesii* complex-like ancestors in central China and *A. yuanbaoshanensis*–*A. fanjingshanensis* from the *A. recurvata* complex-like ancestors in southwest China. However, three populations of *A. ziyuanensis* contained both mitotypes and chlorotypes originating from both regions. For example, two populations (15 and 17) were characterized by mitotype I in the clade with *A. recurvata* from southwest China, while the third population (16) was characterized by mitotype V, which is in the other clade containing *A. fargesii* from central China. Meanwhile, two populations (16 and 17) shared the same chlorotype (A) with *A. fargesii* while the other population (15) had chlorotype I, as did *A. recurvata*. It is obvious that both the mitotype and chlorotype of the eastern population (16) had common origins with *A. beshanzuensis* and *A. fargesii* from central China, while the western population (15) shared common mitotype and chlorotype origins with *A. yuanbaoshanensis*, *A. fanjingshanensis*, and *A. recurvata* from southwest China. However, the central population (17) contained two ancestral lineages based on its different maternal (mitotype) and paternal (chlorotype) origins. Because all populations and individuals of *A. ziyuanensis* are morphologically uniform and distinct from other species (Farjon and Rushforth 1989; Fu et al. 1999; Liu 1971), two possible scenarios may account for the genetic pattern identified for this species. First, *A. ziyuanensis* may be a paraphyletic complex composed by two different lineages. It is evolutionary convergence due to the local environmental constraints that resulted in morphological similarity between different lineages. Second, it is more likely that hybridization or introgressions caused such genetic pattern. For example, this species originated from diploid hybridizations between two different ancestral lineages. Because of the different backcross frequencies, each population may have randomly fixed mitotypes or

Table 2 Genetic diversity estimates for mtDNA and cpDNA variations in six closely related *Abies* species

Regions	H_S	H_T	G_{ST}	N_{ST}
Mitotypes				
Total	0.077 (0.047)	0.616 (0.076)	0.876 (0.069)	0.859 (0.104) ns
SWC	0.000 (0.000)	0.000 (0.000)	0.000 (NC)	0.000 (NC) nc
CTC	0.000 (0.000)	0.000 (0.000)	0.000 (NC)	0.000 (NC) nc
STC	0.000 (0.000)	0.333 (0.222)	1.000 (NC)	1.000 (NC) nc
Chlorotypes				
Total	0.317 (0.068)	0.858 (0.051)	0.631 (0.078)	0.740 (0.070)*
SWC	0.350 (0.120)	0.897 (0.076)	0.609 (0.119)	0.633 (0.135)
CTC	0.566 (0.054)	0.712 (0.062)	0.204 (0.099)	0.262 (NC) nc
STC	0.028 (0.028)	0.739 (0.115)	0.962 (0.036)	0.951 (0.034) ns

Estimates are given for average gene diversity within populations (H_S), total gene diversity (H_T), interpopulation differentiation (G_{ST}), and number of substitution types (N_{ST}) (mean ± SE in parentheses)

ns not significant, *nc* not computed due to small sample size

*p=0.001 (N_{ST} is significantly different from G_{ST} (<))

chlorotypes from paternal or maternal lineages. It is very common in homoploid hybrid species that different populations fixed different chlorotypes or mitotypes from both parental lineages (e.g., Rieseberg et al. 2007; Song et al. 2003). Alternatively, this species originated from one of two ancestral lineages from further north. However, after its establishment, mitotypes or chlorotypes from the other lineage might have introgressed totally or partially into a few populations of *A. ziyuanensis*. These analyses suggested that hybridizations or introgressions between two ancestral lineages that colonized southern China might have occurred during the evolutionary history of *A. ziyuanensis* in response to climatic oscillations.

Our genetic characterization and the inferred interspecific relationships are largely inconsistent with previous studies based on morphological traits (Farjon and Rushforth 1989). Morphologically, *A. fanjingshaensis* and *A. yuanbaoshanensis* are similar and seem to be closely related to *A. fargesii* (all within Sect. *Pseudopicea*) from central China. In addition, *A. beshanzuensis*, *A. ziyuanensis*, and *A. recurvata* are rather similar to one another, together belonging to the same section (Sect. *Momi*). This inconsistency between the morphological and the genetic evidence may suggest that convergent evolution in morphology may have occurred within these species. However, further studies are needed to clarify the underlying mechanisms.

4.3 Intraspecific diversity and conservation

In general, the levels of genetic diversity for mtDNA and cpDNA within the four southern *Abies* species examined (*A. beshanzuensis*, *A. ziyuanensis*, *A. yuanbaoshanensis*, and *A. fanjingshanensis*) were similar (Table S1) but obviously lower than those of other conifers (e.g., Du et al. 2009; Jaramillo-Correa et al. 2008; Tsumura and Suyama 1998). In addition, AMOVA indicated that most of the total variation in mtDNA and cpDNA makers occurred among species or among populations of each species (Table 1). The low genetic diversity within populations and high genetic differentiation between populations (Table 2) are also consistent with a recent study of *A. ziyuanensis* based on intersimple sequence repeat and amplified fragment length polymorphism markers (Tang et al. 2008). This could result mainly from strong genetic drift associated with the presence of small isolated populations during past climatic oscillations after the establishment of these species (Avise 2004). The four southern species examined have been classified as being endangered (Frankham et al. 2002). Unfortunately, habitats suitable for these species continue to deteriorate (Xiang 2001). The total number of surviving individuals for each population/species is still decreasing according to our field investigations. Because of the extremely endangered status of each species, every individual (including those rare seedlings) from any population should be rigorously protected.

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