Genetica (2009) 137:201–212
DOI 10.1007/s10709-009-9386-7

Plastid DNA variation in highly fragmented populations of *Microbiota decussata* Kom. (Cupressaceae), an endemic to Sikhote Alin Mountains

Elena V. Artyukova · Marina M. Kozyrenko · Peter G. Gorovoy · Yury N. Zhuravlev

Received: 11 February 2009 / Accepted: 7 July 2009 / Published online: 24 July 2009

Abstract *Microbiota decussata* Kom. (Cupressaceae) is a subalpine species endemic to the Sikhote Alin Mountains with populations scattered throughout the range. We used sequence data for four noncoding regions of chloroplast DNA to characterize the genetic diversity in populations sampled from different parts of *M. decussata* natural range. No variation was observed in the *trnT–trnF* region, whereas the *trnH–psbA*, *trnS–trnfM*, and *trnS–trnG* regions showed polymorphisms. At the species level, we found a low nucleotide diversity ($\pi = 0.0009$) and high haplotype diversity ($h = 0.981$) as well as high differentiation ($\Phi_{ST} = 0.420$). N_{ST} and G_{ST} values suggested the existence of a phylogeographic structure in *M. decussata*. The observed patterns of diversity could be explained in part by ecological features of the species and its long-term persistence throughout the range with population expansion, successive fragmentation and isolation.

Keywords *Microbiota decussata* · Endemic · Fragmented populations · Genetic structure · cpDNA intergenic regions

Introduction

Microbiota decussata Kom. is a coniferous shrub of the family Cupressaceae (sensu lato). This ancient group is known in the fossil record since the Jurassic, which diversified to fill diverse ecological niches and achieved a wide distribution throughout the world during the Cretaceous and Palaeogene (Stockey et al. 2005). The fossil twigs with leaves or/and reproductive organs of Cupressaceae sensu stricto often occur in the Cretaceous/Tertiary deposits of Sikhote Alin (e.g. Akhmetiev 1973, 1988; Pimenov 1990; Volynets 2005), but there is no evidence of *Microbiota* Kom. The Sikhote Alin palynofloras also contain pollen of Cupressaceae (e.g. Bolotnikova 1979; Pavlyutkin et al. 2005), but the cupressaceous pollen grains could not be distinguished beyond the family level. Our knowledge of the palaeontological history of *M. decussata* is restricted only to the fossil woods. The fossil species *Cupressinoxylon microbiotoides* Blokhina from the Eocene/Oligocene deposits of Yuri Island of the Kurils is most similar to *M. decussata* in the wood structure and is supposed to be a member of the genus *Microbiota* (Blokhina 1988). The only fossil record of *M. decussata* is the well-preserved wood found in the Pliocene Pavlovka lignite field (Bondarenko 2006). Pavlyutkin et al. (2005) however, are of opinion that the age of this lignite field is to be late Eocene through late Oligocene. Based on both molecular and morphological data, *Microbiota* appears most closely related to another monotypic genus *Platycladus* Spach (=*Biota* D. Don), with *P. orientalis* native to northwestern China and North Korea (Blokhina 1988; Jagel and Stützel 2001; Little et al. 2004), however, its wood anatomical features are more primitive than those of the genera *Platycladus*, *Thuja* L., *Thujopsis* Sieb. et Zucc., and *Libocedrus* Endl. (Blokhina 1979). The fossil wood of *M. decussata* was found at Pavlovka together with the woods of *Platycladus orientalis* (Bondarenko 2006), indicative of their co-occurrence in the Pliocene/Oligocene plant associations of Sikhote Alin. Climatic fluctuations in

E. V. Artyukova (✉) · M. M. Kozyrenko · Y. N. Zhuravlev
Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, 690022 Vladivostok, Russia
e-mail: artyukova@biosoil.ru

P. G. Gorovoy
Pacific Institute of Bioorganic Chemistry, Far East Branch of Russian Academy of Sciences, 690950 Vladivostok, Russia

the Pleistocene caused extinction of many species and restriction in the ranges of the taxa that survived throughout Quaternary glacial cycles (Davis and Shaw 2001; Hewitt 2000, 2004; Manchester et al. 2009). Today many genera are monotypic with disjunct or relictual distributions.

M. decussata is the sole species in *Microbiota*, which is the only Cupressaceae genus endemic to the Sikhote Alin Mountains. *M. decussata* is cited as endangered in the Red Data Book of the Primorsky Kray (2008), and as least concern in IUCN 2008 (IUCN Red List of Threatened Species; available from http://www.iucnredlist.org). First described from Mt. Livadiiskaya (Komarov 1923), this species is very restricted in distribution with the natural range stretching from 43°00′ N to 48°50′ N, from the subalpine zone of the southeast Primorskii Krai (=Primorye) to the mountains in the Anyuy River basin in the Khabarovskii Krai (Fig. 1). The known locations of *M. decussata* populations have been described in several summaries, indicative of the habitat positions, which are often barely accessible (Gorovoy and Gurzenkov 1974; Koropachinskii 1989; Vyshin 1990). *M. decussata* has a strongly disjointed distribution; the greatest disjunction of about 300 km occurs between the northern populations growing on the western slope of Mt. Ko in the Chor River basin and on the mountains in the Anyuy River basin. In the southern and middle Sikhote Alin, the populations of *M. decussata* occur mainly at the distinct mountain heights separated by forest habitats. In 1998, one of the authors of the present study (PGG) has found populations of *Microbiota* at the summit of Mt. Snezhnaya of the middle Sikhote Alin (Fig. 1), and a new population has been recently detected in the Chor River basin (Melnikova and Machinov 2004).

M. decussata is a heliophilous prostrate shrub that inhabits the steep stony slopes and screes at altitudes from ca. 300 to 1,700 m above sea level (mainly the timberline or above it). Often forming monodominant communities (Krestov and Verkholat 2003), the species is an anemophilous monoecious plant (Zamyatnin 1963) with ovulate cones containing only a single 2 mm long naked seed. The seeds are able to disperse to a very short distance dropping near the parent plant and germinating within populations almost exclusively after the fires (Urusov 1979). It has been supposed that such traits, as a long life span, long pre-reproductive phase, long-term survival of seeds in soil seed banks, a capability to spread by layering and creeping at free habitats, as well as a broad range of drought and temperature resistance and soil-forming capacity might facilitate the persistence of *M. decussata* throughout the range from the Oligocene (Kurentsova 1968; Urusov 1979). However, it was predicted that ongoing global climate changes would result in the upward

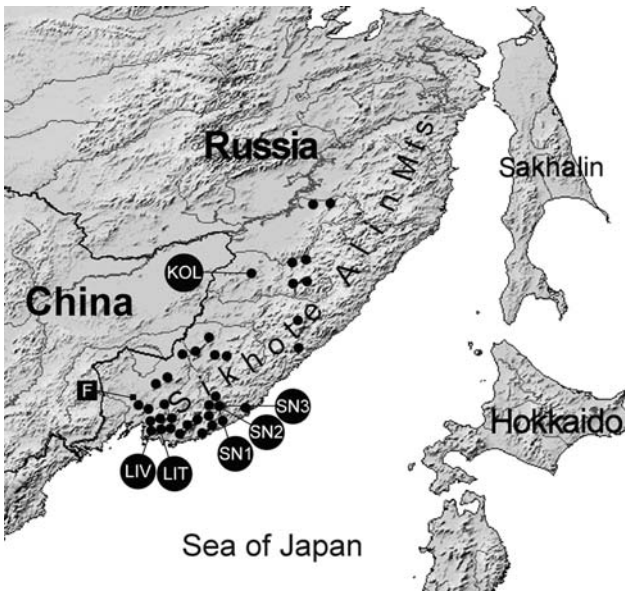

Fig. 1 Distribution of *Microbiota decussata* and collection sites for this study. *Small circles*, population locations according to Urusov (1979) and Koropachinskii (1989); sampled populations: LIT, LIV, SN1, SN2, SN3, KOL (for codes, see Table 1); F, fossil record of the species at Pavlovka

shifts of the timberline and the species movement upward in elevation (Walther et al. 2005) that might lead to loss in habitats and reduce *M. decussata* to a more endangered state.

The analyses of plant cell genomes with different modes of inheritance allow the estimation of forces that have shaped the population structure and can provide information about the evolutionary history of plant populations (Ouborg et al. 1999; Hwang et al. 2003; Zhang et al. 2005). The knowledge of the species history is necessary for evaluating species responses to ongoing climate change (Petit et al. 2008). To reconstructing the species history, evidences from fossil data are desirable. In *Microbiota*, fossil records are poor; and the study of genetic variation in disjunct populations of *M. decussata* can help to verify the putative history of the species and to predict how populations will respond to future environmental changes. In earlier study, we have detected the high level of genetic variation in *M. decussata* using random amplified polymorphic DNA (RAPD) markers that usually represent nuclear genome ($P_{95} = 64\%$, $H_e = 0.254$; Artyukova et al. 2006). In this study, we used sequence data for four noncoding regions of chloroplast DNA (cpDNA) to characterize the genetic variation of plastid genome, for which paternal inheritance has been demonstrated in some other Cupressaceae species studied (e.g. Neale et al. 1989; Kondo et al. 1998; Hwang et al. 2003). We addressed the following questions: (1) what level of genetic variability is in this species; (2) how genetic variation is distributed

within and among populations; (3) what forces have shaped the population structure; and (4) how the species will respond to ongoing climate change. Our results indicate two main phases in the evolution of the species. At first, the ancestral population expanded the range throughout the Sikhote Alin area during dry, cool climate and glaciations of the Pleistocene. Within the Holocene, this population had been fragmented in consequence of shifting climatic zone and supplanting by temperate flora that resulted in the disjunct present-day distribution. Durative invasion of the natural *M. decussata* habitats by temperate species enhanced by the climate warming can bring about rapid contraction of the extant populations and extinction of the species before its genetic collapse.

Materials and methods

Population sampling

Six populations of *Microbiota decussata* were sampled from different parts of the Sikhote Alin Mountains (Fig. 1). The two samples from populations of the southern Sikhote Alin were sampled from Mt. Litovka (LIT) and Mt. Livadiiskaya (LIV) of the Livadiiskii Range, approximately 10 km distant from each other. The three small populations (SN1, SN2 and SN3) sampled in the middle Sikhote Alin are located on Mt. Snezhnaya, only about 0.3 km from each other. The northernmost population (KOL) grows at the steep slopes of the Bolshaya Kolomi River valley (Chor River basin) in the northwestern branches of the Sikhote Alin (Melnikova and Machinov 2004). Randomly selected plants approximately 100 m apart were sampled in each population. Sample size and the geographic coordinates for each population are given in Table 1.

DNA amplification and sequencing

Total DNA was extracted from leaves according to the protocol described by Isabel et al. (1993). To investigate cpDNA variation in *M. decussata*, we sequenced four noncoding intergenic spacer regions of the chloroplast genome: the *trnH*^{GUG}–*psbA*, the *trnS*^{UGA}–*trnfM*^{CAU}, the *trnS*^{GCU}–*trnG*^{UUC}, and the *trnT*^{UGU}–*trnF*^{GAA}. Double-stranded templates for direct sequencing were amplified on a thermal cycler UNO II 48 (Biometra, Germany) using the PCR parameters and universal primer pairs (Table 2) that were recommended for the regions tested by Shaw et al. (2005). The PCR products were sequenced using a BigDye terminator v. 3.1 sequencing standard kit (Applied Biosystems, USA). Sequencing was carried out in both directions under cyclic sequencing conditions described by Shaw et al. (2005) and with the same pairs of primers as

Table 1 Sampling site locations, codes, sample size (*n*) and voucher specimens of *Microbiota decussata* populations

Region	Population	Code	Latitude (N)	Longitude (E)	Altitude (m)	*n*	Voucher specimens[a]
South Sikhote Alin	Litovka Mountain, screes near the summit	LIT	43°07′12″	132°45′58″	1,100–1,200	10	VLA148552–VLA148561
	Livadiiskaya Mountain, screes at the west slopes	LIV	43°04′00″	132°41′00″	700–800	10	VLA148562–VLA148571
Middle Sikhote Alin	Snezhnaya Mountain, screes near the summit	SN1	43°44′10″	134°26′40″	1,600	6	VLA148594–VLA148599
	Snezhnaya Mountain, screes near the summit	SN2	43°44′19″	134°26′40″	1,600	6	VLA148582–VLA148587
	Snezhnaya Mountain, screes near the summit	SN3	43°44′38″	134°26′40″	1,600	6	VLA148588–VLA148593
Northern Sikhote Alin	Bolshaya Kolomi River, steep slopes of the valley	KOL	47°16′00″	135°58′ 00″	340	10	VLA148572–VLA148581

[a] All voucher specimens are deposited at the Herbarium of Institute of Biology and Soil Science, Far Eastern Branch of Russian Academy of Sciences (IBSS FEBRAS), Vladivostok

Table 2 Primers cited from Shaw et al. (2005), fragment size, and GenBank accession numbers for sequences of the four chloroplast regions used in this study

CpDNA region	Nucleotide sequence (5′ → 3′)		Fragment size, bp	GenBank accession number
	Forward primer	Reverse primer		
trnH^{GUG}–*psbA*	GCGCATGGTGGATTCACAATCC[a]	GTTATGCATGAACGTAATGCTC[a]	512	AM887665–AM887666, FM205067–FM205112
trnS^{GCU}–*trnG*^{UUC}	AGATAGGGATTCGAACCCTCGGT[a]	GTAGCGGGAATCGAACCCGCATC[a]	1,640–1,645	AM887668–AM887682, FM205156–FM205188
	GGCGGGTATAGTTTAGTGGTAAAA[b]	TTTTACCACTAAACTATACCCGC[b]		
	AAGGTAAGGGACGGAGTG[b,c]	CCGTCCCTTACCTTGTTG[b,c]		
trnS^{UGA}–*trnfM*^{CAU}	GAGAGAGAGGGATTCGAACC[a]	CATAACCTTGAGGTCACGGG[a]	862	AM887660–AM887665, FM205113–FM205155
		GTAGCGGGAATCGAACCCGCATC[b]		
trnT^{UGU}–*trnF*^{GAA}	CATTACAAATGCGATGCTCT[a]	ATTTGAACTGGTGACACGAG[a]	1,338	AM887667, FM205189–FM205235
	TCTACCGATTTCGCCATATC[b]	TCTACCGATTTCGCCATATC[b]		
	GGTTCAAGTCCCTCTATCCC[b]	GGGGATAGAGGGACTTGAAC[b]		

[a] Primer both for PCR amplification and for cyclic sequencing

[b] Internal primer for cyclic sequencing only

[c] Primer designed for this study

those used for amplification. In addition, internal primers were used for sequencing the all regions except for the *trnH–psbA* (Table 2). The sequences were analyzed on an ABI PRISM 310 sequencer (Applied Biosystems, USA). Complete sequences were assembled using the Staden Package v. 1.4 (Bonfield et al. 1995) and aligned manually with the program SeaView (Galtier et al. 1996). All DNA fragments that contained substitutions and/or microsatellite variants were retested (reamplified and resequenced) to verify that our results were repeatable.

Data analysis

For each individual, sequence data for the four regions were combined. Two mononucleotide repeats revealed within the *trnS–trnG* region were included in the data set since repeatability tests allowed us to exclude PCR errors. Site mutations and indels were assumed to evolve with equal possibility although they may exhibit different mutation rates. Rate of plastid DNA evolution is very slow, and differences between site and indel mutation rates are unlikely to affect the resolution of intraspecific phylogenetic relationships. We treated indels as a fifth state interpreting each increase or decrease of a single repeat unit as a single mutational event (Simmons and Ochoterena 2000). The analyses of molecular variation were carried out using the software Arlequin v. 3.11 (Excoffier et al. 2005) and DnaSP v. 4.5 (Rozas et al. 2003). We computed numbers of haplotypes and values of haplotype diversity (h) and nucleotide diversity per site (π). To detect departures from the standard neutral model of evolution, we performed Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) tests using Arlequin and Fu and Li's (1993) D^* and F^* tests using DnaSP. These tests show different degrees of sensitivity to deviation from neutrality caused by demography or selection. However, their inferences may be ambiguous because different processes can produce the same patterns (Fu 1997). The significance levels of the tests were assessed by generating 10,000 random samples and using model-based simulations (Excoffier et al. 2005). Significant positive values of Tajima's D, Fu and Li's D^* and F^* tests and insignificant values of Fu's F_S test would suggest either balancing selection, or a reduction in population size in the recent past while significant values only for Fu's F_S suggest population growth or hitchhiking (Fu 1997). To test for population demographic changes, a mismatch pairwise distribution analysis (MDA) was also performed using Arlequin. We tested the fit of the observed mismatch distributions to a model by coalescent simulations of 10,000 samples with the sum of squared deviations between observed and expected mismatch distributions (*SSD*) and raggedness index (r) as test statistics. The distribution is multimodal in populations of constant size

while unimodal patterns with the low insignificant values of *SSD* and *r* are typical for expanding populations (Rogers and Harpending 1992).

An analysis of molecular variance (AMOVA; implemented in Arlequin) was computed to estimate the partition of the genetic variation within and between populations and the values of pairwise genetic distances (F_{ST}) between populations. The significance of the variance components was determined with a permutation test (10,000 replicates). Thresholds of significance for pairwise F_{ST} were estimated with the Bonferroni correction for multiple tests. We used Mantel test with 10,000 permutations to analyze the relationships between the matrices of genetic differentiation defined as F_{ST} or the linear function $F_{ST}/(1 - F_{ST})$ (Rousset 1997) and geographic distances using Arlequin. To compute and compare the differentiation indices N_{ST} and G_{ST}, we used the software Permut v. 2.0 (available from http://www.pierroton.inra.fr/genetics/labo/Software) with a permutation test (10,000 permutations). N_{ST} takes into account the similarities of haplotypes whereas G_{ST} is based solely on the haplotypes frequencies. The higher value for N_{ST} than G_{ST} would be indicative of a phylogeographic structure (Pons and Petit 1996; Petit et al. 2005).

To determine relationships among haplotypes and factors that may have influenced these relationships, we employed nested clade analysis (NCA) using the program ANeCA v. 1.1 (Panchal 2007). Networks are considered the most appropriate way to represent the relationships within a species (Schaal et al. 2003). To construct a network of haplotypes and a cladograph showing the nesting structure of the haplotypes, we carried out statistic parsimony analysis with a 95% confidence limit for parsimony using the TCS program (Clement et al. 2000) as implemented in the software package ANeCA. This software package also includes GeoDis v. 2.2 (Posada et al. 2000) for calculating the various nested clade analysis distance measures and their statistical significances; and the inference key dated November, 2005 to interpret the results in an automated fashion. There has been recent discussion about the validity of nested clade analysis and its tendency to false positives (Beamont and Panchal 2008; Garrick et al. 2008; Petit 2008; Templeton 2008). However, it remains one of the common methods to describe an association between haplotype variation and geography and infer possible species history especially in conjunction with other analytical approaches (e.g. Zhang et al. 2005; Bartish et al. 2006; Garrick et al. 2008; Sosa et al 2009).

Results

The *trnH–psbA*, the *trnS–trnfM*, the *trnS–trnG* and the *trnT–trnF* regions of cpDNA were successfully sequenced for 48 plants from different populations of *M. decussata*. A total of 4,357 bp of aligned chloroplast sequences were obtained, comprising 512, 862, 1,645, and 1,338 bp for the *trnH–psbA*, the *trnS–trnfM*, the *trnS–trnG*, and the *trnT–trnF*, respectively. All regions studied were rich in adenine and thymine, with contents between 65.1% (*trnT–trnF*) and 70.3% (*trnS–trnG*). In total, 14 polymorphic sites were detected (Table 3). We found one polymorphic site within the *trnH–psbA* (position 487) and three nucleotide substitutions within the *trnS–trnfM* region (positions 10, 122 and 770), while ten sites showed polymorphism for the *trnS–trnG* region: two nucleotide substitutions (positions 381 and 525); a two base-pair indel (between sites 1610 and 1613); and two mononucleotide repeats: a poly-A motif, repeated 10 or 11 times (position 568) and the poly-T repeat, which varied in length between 11 and 16 bp (positions 305–309). For the *trnT–trnF* region, all the individuals we analyzed had the same sequence. Sequences of the four regions for each individual studied are deposited in GenBank (See Table 2 for accession numbers).

Preliminarily, we analyzed two data sets (with and without poly-A and poly-T repeats) to evaluate the robustness of the results. Most analyses showed consistent results with the lower values of some parameters for data set without the mononucleotide repeats. Such mononucleotide repeats had been shown to provide valuable information in intraspecies studies (Rendell and Ennos 2003; Walter and Epperson 2005; Nagiri and Gaudeul 2007). Therefore, only the results based on data with poly-A/T, which increased the distance between haplotypes, are presented here. In the combined data set of four noncoding regions, the 14 polymorphic site combinations produced 34 haplotypes among the 48 individuals studied (Table 3). Twenty seven haplotypes were only represented in single individuals, and only three haplotypes were found in more than one population. SN1 and LIV shared haplotype H3, haplotype H4 was shared by SN1, SN2 and KOL, and haplotype H11 was shared by SN2 and LIT. Genetic diversity estimates at population and species levels are summarized in Table 4. In *M. decussata*, the high level of haplotype diversity along with the low nucleotide diversity was observed at the species level and within populations with a higher value of haplotype diversity in KOL and higher values of nucleotide diversity in SN1 and KOL.

The network construction by TCS yielded a single network with one mutational step between any two haplotypes (except S2 and S7), and only three missing haplotypes (Fig. 2). The network showed the presence of loops due to more than one parsimonious connection of a haplotype to the rest of the network that could indicate homoplasy caused by recurrent mutations at the same sites, but recombination could not be ruled out also. Haploid and uniparentally inherited cpDNA is usually thought to be a

Table 3 Variable sites in the *trnH–psbA*, *trnS–trnG* and *trnS–trnfM* chloroplast spacer regions and the distribution of haplotypes in *Microbiota decussata* populations

Haplotype[a]	Nucleotide position														Haplotype distribution[b]					
	trnH–psbA	*trnS–trnG*										*trnS–trnfM*								
	487	305	306	307	308	309	381	525	568	1611	1612	10	122	770	LIT	LIV	KOL	SN1	SN2	SN3
S1	A	T	T	T	T	T	C	T	A	A	A	A	A	T				1		
S2	A	T	T	–	–	–	C	T	A	–	–	A	A	T				1		
H3	A	–	–	–	–	–	T	T	A	A	A	A	A	T		2		2		
H4	A	T	T	T	T	–	C	T	A	A	A	A	A	T			1	1	1	
S5	A	T	T	T	–	–	T	T	A	A	A	A	A	T				1		
S6	A	T	T	T	–	–	T	T	–	A	A	A	A	T						3
S7	A	T	T	T	T	–	C	T	A	–	–	A	A	T						1
S8	A	T	T	T	–	–	C	T	–	A	A	A	A	T						1
S9	A	T	T	T	T	–	T	T	A	A	A	A	A	T						1
S10	C	T	T	T	T	–	T	T	A	A	A	A	A	T					1	
H11	C	T	T	T	–	–	C	T	A	A	A	A	A	T	2				1	
S12	A	T	T	T	–	–	C	T	A	A	A	A	A	T					3	
Lt13	C	T	T	T	–	–	C	T	A	A	A	T	G	T	1					
Lt14	C	T	T	T	–	–	C	T	A	A	A	A	G	T	2					
Lt15	C	T	T	T	–	–	C	T	A	A	A	T	G	A	1					
Lt16	A	T	T	T	–	–	C	T	A	A	A	A	G	A	1					
Lt17	C	T	T	T	–	–	C	T	A	A	A	A	G	A	1					
Lt18	C	T	T	T	T	–	C	T	A	A	A	A	G	T	1					
Lt19	A	T	–	–	–	–	C	T	A	A	A	A	G	T	1					
Lv20	C	T	–	–	–	–	T	T	A	A	A	A	G	T		1				
Lv21	A	–	–	–	–	–	C	T	A	A	A	A	G	T		1				
Lv22	A	–	–	–	–	–	T	T	A	A	A	A	G	A		1				
Lv23	A	–	–	–	–	–	T	T	A	A	A	A	G	T		3				
Lv24	C	–	–	–	–	–	T	T	A	A	A	A	G	T		1				
Lv25	A	–	–	–	–	–	T	T	A	A	A	T	G	A		1				
K26	A	T	T	–	–	–	C	T	A	A	A	A	A	T			1			
K27	A	T	T	T	T	–	C	G	A	A	A	A	A	T			1			
K28	A	T	–	–	–	–	C	T	A	A	A	A	A	T			1			
K29	A	T	T	T	–	–	C	T	A	A	A	A	G	T			1			
K30	C	T	T	T	T	–	C	G	A	A	A	A	G	T			1			
K31	A	T	T	–	–	–	C	T	A	A	A	A	G	T			1			
K32	A	T	T	T	–	–	C	T	A	A	A	T	G	A			1			
K33	A	T	T	–	–	–	C	T	A	A	A	T	G	T			1			
K34	C	T	T	–	–	–	C	T	A	A	A	A	G	A			1			

–, deletion

[a] Haplotypes private to populations LIT, LIV, KOL and three populations from Snezhnaya Mt. are denoted as Lt, Lv, K, and S, respectively; haplotypes shared by several populations are denoted as H

[b] For code of populations, see Table 1

single 'locus' that is nonrecombining (Petit et al. 2005). However, recombination events have been detected in the cpDNA of some plants (Marshall et al. 2001; Shiraishi et al 2001; Hamilton et al. 2003; Hale et al. 2004; Nagiri and Gaudeul 2007). After resolving one loop according to the published rules (Crandall and Templeton 1993), the network was partitioned into 17 one-step clades, seven two-step clades, and three three-step clades using ANeCA. Clade 3-1 was formed mainly by haplotypes of the populations SN1, SN2 and SN3 (except for H3), and clades 3-2 and 3-3 comprised the majority of haplotypes from LIV and LIT, respectively, whereas all the haplotypes from

Table 4 Diversity parameters and test statistics for *Microbiota decussata* populations based on the four chloroplast intergenic spacer combined sequences

Population	Sample size	*S*	*nh*	*h* (SD)	π (SD)	Neutrality tests				Population size tests	
						D	*D**	*F**	F_S	*SSD*	*r*
LIT	10	7	8	0.956 (0.059)	0.00050 (0.00034)	0.32418 ns	1.23914 ns	1.13716 ns	−4.81943**	0.00958 ns	0.08889 ns
LIV	10	10	7	0.911 (0.077)	0.00043 (0.00031)	−0.68235 ns	−0.02396 ns	−0.20753 ns	−3.89094**	0.01935 ns	0.13432 ns
SN1	6	8	5	0.933 (0.122)	0.00088 (0.00060)	1.05247 ns	1.15768 ns	1.44510 ns	−0.83934 ns	0.03482 ns	0.12000 ns
SN2	6	3	4	0.800 (0.172)	0.00033 (0.00027)	0.06221 ns	0.03984 ns	−0.05002 ns	−1.15958 ns	0.01285 ns	0.12000 ns
SN3	6	5	4	0.800 (0.172)	0.00053 (0.00039)	1.05247 ns	1.02905 ns	0.85057 ns	−0.27174 ns	0.01785 ns	0.06667 ns
KOL	10	9	10	1.000 (0.045)	0.00083 (0.00052)	0.06757 ns	0.77491 ns	0.67454 ns	−7.70585**	0.00297 ns	0.05728 ns
Species level	48	14	34	0.981 (0.008)	0.00092 (0.00052)	1.10591 ns	1.17785 ns	1.35375 ns	−25.92863**	0.00080 ns	0.01832 ns

S number of polymorphic sites, *nh* number of haplotypes, *h* haplotype diversity, π nucleotide diversity, *SD* standard deviations, *D* Tajima's *D*-test, *D** Fu and Li's *D**-test, *F** Fu and Li's *F**-test, F_S Fu's F_S-test, *SSD* the sum of squared deviations, *r* raggedness index

Significance levels are based on 10,000 coalescent simulations under an infinite-site neutral model of evolution: ** $P < 0.00001$, *ns* not significant ($P > 0.10$). For population code, see Table 1

KOL were divided between these three clades. The nested contingency analysis indicated no significant geographic associations within clades 3-1 and 3-3 and at all but one low nesting levels (one- and two-step clades). Only the one-step level nested in clade 2-6 ($\chi^2 = 31.56$; $P = 0.007$) and the two-step level nested in clade 3-2 ($\chi^2 = 13.06$; $P < 0.001$) as well as the total cladogram ($\chi^2 = 51.25$; $P < 0.001$) showed significant levels of geographic association. The inference key suggested contiguous range expansion for the total cladogram corresponding to the oldest historical event. Restricted gene flow with isolation-by-distance was inferred for the two-step level nested in clade 3-2. For clade 2-6, we obtained an inconclusive outcome because the tip-interior status could not be determined (Fig. 2).

The comparison of two differentiation indices (G_{ST} and N_{ST}) also indicated a nonrandom distribution of cpDNA haplotypes. The values of G_{ST} and N_{ST} were 0.090 ± 0.038 and 0.365 ± 0.068, respectively, and the difference between these two parameters was significant ($P < 0.01$). The AMOVA gave concordant result showing that there are the high amounts of variation both within and between populations with 58.01 and 41.99% of the total cpDNA variance, respectively ($P < 0.0001$; Table 5). The values of pairwise F_{ST} among pairs of populations ranged from 0.1170 to 0.6442, with most comparisons (11 out of 15) being significantly different from zero ($P < 0.03$; Table 6). The four non-significant comparisons involved populations from Mt. Snezhnaya. Pairwise F_{ST} values between other pairs of populations were significant and just two pairs (LIT vs. KOL and KOL vs. SN2) showed values that were no longer significant after Bonferroni correction for multiple testing (Table 6). Examination of pairwise F_{ST} values between populations indicated that the population LIV was the most different from the others with the pairs of populations SN1 and KOL and LIT and KOL being more similar. The Mantel tests showed that there was no a significant effect of isolation by distance because the matrix of geographic distances was not significantly correlated to F_{ST} or the linearized F_{ST} ($r_M = -0.653$; $P = 0.870$ and $r_M = -0.667$; $P = 0.964$, respectively).

Demographic events leave traces on the patterns of genetic diversity in populations, and tests of neutrality and MDA could indicate the likely mechanisms that are responsible for the observed cpDNA polymorphism. Significance of tests for deviations from the standard neutral expectations varied depending on statistics. Values of Tajima's *D*, Fu and Li's *D** and *F** tests were not significantly different from zero ($P > 0.10$; Table 4) for all populations and for the entire data set (all samples pooled). Values of Fu's F_S test were negative for all populations and for the entire data set. The populations SN1, SN2 and SN3 had negative F_S values that were not significantly different

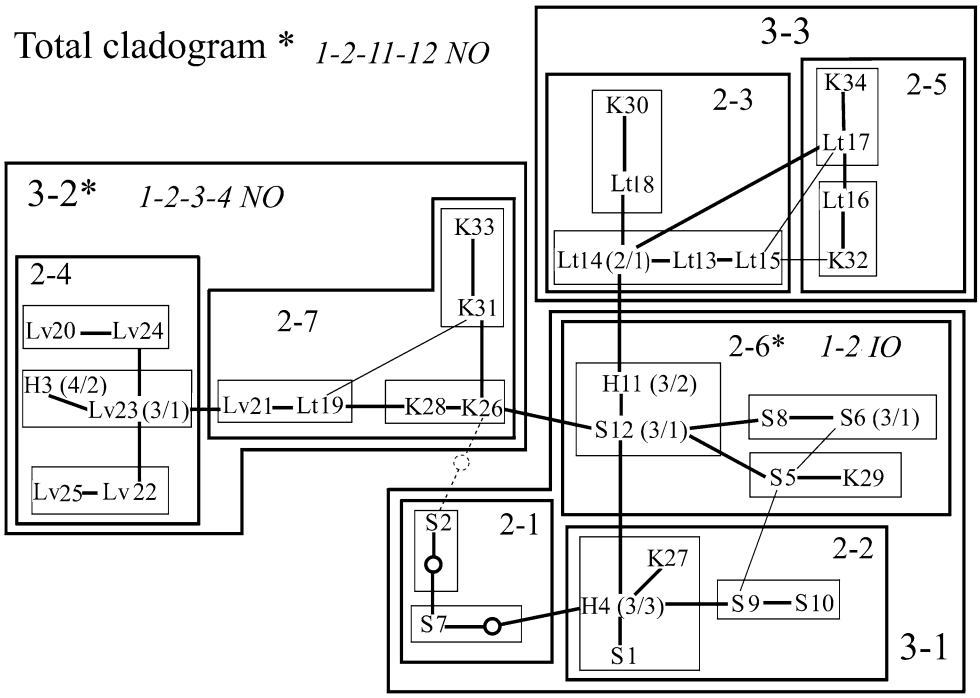

Fig. 2 Statistical parsimony (95%) network showing nested clades for cpDNA haplotypes identified in *Microbiota decussata*. Haplotypes are designated by names defined in Table 3. Small *empty circles* indicate missing haplotypes that were not sampled in our study. *Solid line* connecting haplotypes represents a single mutational change. *Thin lines* represent the alternative ambiguous connections, and *dotted lines* indicate connections had been resolved as per Crandall and Templeton (1993). *Thin-lined boxes* enclose one-step clades, *thicker-lined boxes* enclose two-step clades, which are designated by "2-*n*" and three-step clades ("3-*n*"), where *n* is a number assigned to identify the clade. The numbers in parentheses after the haplotype names (except for those that occurred in a single individual) indicate the number of individuals representing a given haplotype/the number of populations in which this haplotype occurred. *Stars* indicate the clades in which geographic associations are significant with the nested contingency analysis ($P < 0.05$) and for which the chains of inference are shown: *1-2 IO*—inconclusive outcome; *1-2-3-4 NO*—restricted gene flow with isolation by distance; *1-2-11-12 NO*—contiguous range expansion

Table 5 Analyses of molecular variance (AMOVA) for *Microbiota decussata* populations based on the four chloroplast intergenic spacers combined sequences

Source of variation	d.f.	CV	% Total	Fixation index
Among populations	5	0.877	41.99	$\Phi_{ST} = 0.420$*
Within populations	42	1.256	58.01	
Total	47	2.133		

d.f. degrees of freedom, *CV* variance-component estimates, *% Total* percentage of total variance contributed by each component, Φ_{ST} correlation within populations relative to the total

* $P < 0.0001$. Significance levels are based on 10,000 permutations

from zero ($P > 0.05$) due to less power of Fu's F_S test for small sample sizes (Ramos-Onsins and Rozas 2002). For the other populations and for the entire data set, Fu's F_S was strongly significant ($P < 0.0001$) suggesting population growth or hitchhiking (Fu 1997). The results of MDA were consistent with the population expansion model. The entire data set showed a clearly unimodal distribution pattern fitting a model of population growth (Fig. 3). Mismatch distribution plots for all populations also had unimodal patterns and *SSD* and *r*-index values that did not reject an expansion model (Table 4).

Discussion

In general, plants with fragmented ranges show low levels of genetic variation within populations and high levels of divergence of populations. Population isolation leads to low gene flow between populations, which increases the effects of genetic drift in small populations and might result in reduction of genetic diversity which in turn leads to a decrease in its hardiness and fitness to adapt to environmental changes (e.g. Reed and Frankham 2003; Jump and Peñuelas 2006). Previously using RAPD markers (Artyukova et al. 2006), we detected the high level of genetic variation in *M. decussata* that was similar to the levels found in some woody plants with fragmented or restricted ranges by studying dominantly inherited markers (Hwang et al. 2001; Bekessy et al. 2002; Allnutt et al. 2003; Renau-Morata et al. 2005; Hao et al. 2006). By studying the sequences of four cpDNA noncoding regions,

Table 6 Pairwise F_{ST} values between populations of *Microbiota decussata*

Population	LIT	LIV	KOL	SN1	SN2
LIV	0.64419**				
KOL	0.12243*	0.49149**			
SN1	0.39204**	0.40892**	0.11008 ns		
SN2	0.30992**	0.69912**	0.12500*	0.11698 ns	
SN3	0.49504**	0.66267**	0.27805**	0.13333 ns	0.23256 ns

Significance levels are based on 10,000 permutations

ns not significant ($P > 0.05$)

* Significant at $P < 0.05$ and not significant after Bonferroni multiple test corrections ($P > 0.008$)

** Significant after Bonferroni multiple test corrections ($P < 0.001$). For code of populations, see Table 1

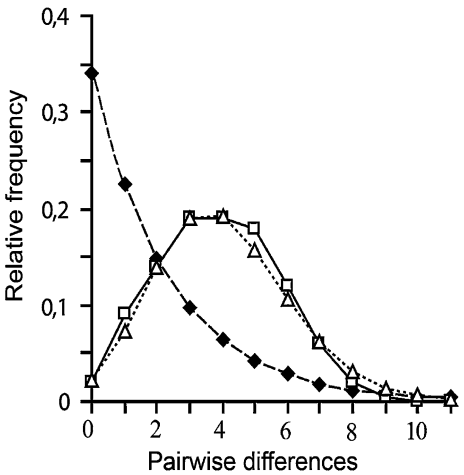

Fig. 3 Mismatch distribution of pairwise nucleotide differences between haplotypes observed in *Microbiota decussata* (*open square*). The distributions fitted to the data under a model of population expansion (*open triangle*) and model of constant population size (*filled square*) are also represented

we found that there is the considerable level of haplotype diversity along with the low level of nucleotide diversity in *M. decussata* (Table 4). The level of cpDNA variation in *M. decussata* is congruous to that in some other woody species, such as *Pinus sylvestris* (*h*, 0.950–0.987; Provan et al. 1998), *Cyclobalanopsis glauca* (π, 0.00065; *h*, 0.68; Huang et al. 2002), *Cunninghamia konishii* and *C. lanceolata* (π, 0.00190 and π, 0.00176, respectively; Hwang et al. 2003), and *Pinus nigra* (*h*, 0.939–1.00; Afzal-Rafii and Dodd 2007). A large number of haplotypes with low nucleotide diversity found in *M. decussata* may be a consequence of homoplasy or may result from rapid population growth of an ancestral population with a low effective population size (Abramson 2007).

Our analyses show that genetic diversity in *M. decussata* is partitioned among the population sites. High differentiation of populations in *M. decussata* is similar to those of some long-lived, wind-pollinated species with fragmented distributions (Huang et al. 2002; Petit et al. 2005; Jaramillo-Correa et al. 2006; Provan et al. 2007). The significantly higher value for N_{ST} than G_{ST} indicates that closely related haplotypes are more likely to co-exist in the same populations (Pons and Petit 1996; Petit et al. 2005). On the other hand, we found the lack of a relationship between genetic and geographic distances, which suggests that the differentiation had not occurred under the isolation-by-distance model. Differentiation in *M. decussata* appears to be associated with historical events and complex mountain topography of the range. In fact, the genetically similar populations (LIT and KOL, and SN1 or SN2 and KOL) are geographically distant that may reflect a footprint of historical, rather than contemporary, gene flow and slow genetic drift. The presence of the same substitutions and shared haplotypes in populations from the opposite parts of the range indicates that at one time, ancestral populations of this species might have contiguous distribution range. The large number of unique, closely related haplotypes within each population may suggest that the distribution area of *M. decussata* was fragmented a long time ago by extinction of populations in the adjusted territory. Fragmentation and geographic isolation of remnant populations in concert with very short-distance seed dispersal and the lack of vacant habitats could lead to reduction in gene flow and strong differentiation. The divergence of the population LIV indicates substantial isolation and may be either due to drift, promoted by low levels of historical gene flow and founder effect, or selection in absence of gene flow. However, a severe bottleneck event would result in extinction of some haplotypes, and the genetic network would have a large number of missing haplotypes that separated the observed haplotypes. In contrast, most haplotypes of *M. decussata* are contiguous to one another on the network. The patterns of the observed genetic variation are consistent with the expectations of the neutral equilibrium model of evolution. The results of MDA and neutrality tests (Fig. 3; Table 4) give evidences of population expansion for most populations and at the species level, but selection cannot be ruled out completely

although it is unlikely that selection would strongly affect noncoding regions of cpDNA. Results from the NCA confirm a range expansion only at the species level. The second NCA inference of restricted gene flow with isolation-by-distance for clade 3-2 contradicts the results of Mantel test and may be 'false positive' (Beamont and Panchal 2008) or may reflect a footprint of restricted gene flow between the populations LIV and KOL in the past.

In contrast to intensive investigations on plants of Europe and North America, there is a comparative paucity of phylogeographic data for species of the northeastern Asia (Beheregaray 2008). The fossil data (see above) as well as the fragmented present-day range and biological and ecological aspects of the species allow us to propose (following some other researchers, e.g. Blokhina 1988) that the origin of *M. decussata* could be dated back to the Oligocene. The species probably arose high up in the Sikhote Alin Mountains at the timberline. Three populations from Mt. Snezhnaya studied coincide by position with the assumed place of species origin at the watershed of the ancient Ussuri and Partizanskaya river basins (Urusov 1979). The presence of haplotypes shared with populations from other range regions may indicate an ancient origin. Due to its high tolerance to drought and broad range of temperature, *M. decussata* could expand its range during dry and rather cool periods at the Oligocene/Miocene boundary. During glacial periods of the Pleistocene, when cold adapted mountain plants in Europe expanded their ranges to lowland and were distributed in the territory between the northern ice shield boundary and glaciated mountains (Hewitt 2004), large areas of East Asia were ice free, and the alpine tundra occupied most of the Sikhote Alin Mountains. Arcto-Tertiary species including most of the Cupressaceae formerly occurring in this area vanished or shifted their ranges south (Manchester et al. 2009) while *M. decussata* was able to survive and expand its range upon new free areas (Urusov 1988). Climatic and landscape changes at the Pleistocene–Holocene boundary caused a rise in the timberline, and *M. decussata*, as most mountain plants, retreated towards higher elevations. Enrichment of plant communities of the Sikhote Alin Mountains with temperate species also resulted in the shifting of *M. decussata* stands to the ecotopes with severe climatic and soil conditions (e.g. the steep stony slopes and screens), and in the splitting of the large ancestral population forming the disjunct present-day distribution.

Hence, the genetic diversity found in the modern populations of *M. decussata* can be explained by their origin from a large and long-established population, which had survived the Quaternary glaciations within a permanent range and had not been experiencing severe bottlenecks. However, acceleration in the upward shift of the timberline has been detected in some regions in response to ongoing global climate changes (Moiseev and Shiyatov 2003; Walther et al. 2005; Soja et al. 2007; Truong et al. 2007). This upward movement of timberline, which has forced *M. decussata* to migrate up to mountain summits with taluses (Urusov 1988; Krestov and Verkholat 2003), may lead to the further contraction of remnant populations that could survive under the harsh environmental conditions (e.g. poor soil, high solar radiation, extreme winds), which are unsuitable for other species.

In conclusion, *M. decussata* has the potential for survival in ongoing climate changes due to its physiological and ecological range of tolerance and life history traits that are conducive to the maintenance of the historical genetic diversity. Nevertheless, permanent invasion of the natural *M. decussata* habitats by temperate species enhanced by the climate warming and a lack of free habitat can bring about rapid contraction of the extant population and extinction of the species before its genetic collapse.

Acknowledgements We are grateful to Dr. Alla B. Melnikova and Dr. Valentin V. Yakubov for their assistance in collecting plant material. We also appreciate the helpful comments on this manuscript from two anonymous referees. This work was supported by FEBRAS grants 06-I-P11-028, 06-I-P11-030 and 06-II-CO-05-021; and by grants NSH 6923-2006.4 and NSH 8969-2006.4 "Leading School of Thought" from the President of Russian Federation.

References

Abramson NI (2007) Phylogeography: results, issues and perspectives. VOGiS Herald 11:307–331

Afzal-Rafii Z, Dodd RS (2007) Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (*Pinus nigra*) in western Europe. Mol Ecol 16:723–736

Akhmetiev MA (1973) Sikhote Alin Miocene flora (Botchi River). Nauka, Moscow

Akhmetiev MA (1988) Cenozoic floras of the Eastern Sikhote Alin. Geological Institute of Academy of Sciences of the USSR, Moscow

Allnutt TR, Newton AC, Premoli A, Lara A (2003) Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. Biol Conserv 114:245–253

Artyukova EV, Kozyrenko MM, Reunova GD, Melnikova AB (2006) Genetic diversity in endemic to Sikhote Alin Mountains *Microbiota decussata* Kom. (Cupressaceae) assessed using RAPD analysis. In: Machinov AN (ed) Modern problems of regional development: materials of interregional scientific conference, Khabarovsk, October, 17–20, 2006. Far Eastern Branch of the Russian Academy of Sciences, Khabarovsk, pp 172–174

Bartish IV, Kadereit JW, Comes HP (2006) Late Quaternary history of *Hippophae rhamnoides* L. (Elaeagnaceae) inferred from chalcone synthase intron (*Chsi*) sequences and chloroplast DNA variation. Mol Ecol 15:4065–4083

Beamont MA, Panchal M (2008) On the validity of nested clade phylogeographical analysis. Mol Ecol 17:2563–2565

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Mol Ecol 17(17):3754–3774

Bekessy SA, Allnutt TR, Premoli A et al (2002) Genetic variation in the vulnerable and endemic Monkey Puzzle tree, detected using RAPDs. Heredity 88:243–249

Blokhina NI (1979) Comparative wood anatomy of *Microbiota decussata* and related genera of Cupressaceae. In: Krasilov VA (ed) Far Eastern palaeofloristics. Far Eastern Branch of the Academy of Sciences of the USSR, Vladivostok, pp 20–37

Blokhina NI (1988) Fossil woods. In: Krasilov VA, Blokhina NI, Markevitch VS, Serova MJ (eds) Cretaceous–Palaeogene of the Lesser Kuril Islands. New data on palaeontology and geological history. Far Eastern Branch of the Academy of Sciences of the USSR, Vladivostok, pp 26–40

Bolotnikova MD (1979) Spore-pollen complexes of the tertiary deposits of western coast of the Sea of Japan. Nauka, Moscow

Bondarenko OV (2006) Fossil woods from the Pliocene of Southern Primorye. PhD Thesis, Institute of Biology and Soil Science, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok

Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Res 23:4992–4999

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intra-specific phylogeny reconstruction. Genetics 134:959–969

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. Science 292:673–679

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Fu Y-X, Li W-H (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Galtier N, Gouy M, Gautier C (1996) SeaView and Phylowin, two graphic tools for sequence alignment and molecular phylogeny. Comput Appl Biosci 12:543–548

Garrick RC, Dyer RJ, Beheregaray LB, Sunnucks P (2008) Babies and bathwater: a comment on the premature obituary for nested clade phylogeographical analysis. Mol Ecol 17:1401–1403

Gorovoy PG, Gurzenkov NN (1974) *Microbiota decussata* Kom. In: Tolmachev AI (ed) Endemic high-altitude plants of north Asia. Atlas. Nauka, Novosibirsk, pp. 5, 102

Hale ML, Borland AM, Gustafsson MHG, Wolff K (2004) Causes of size homoplasy among chloroplast microsatellites in closely related *Clusia* species. J Mol Evol 58:182–190

Hamilton MB, Braverman JM, Soria-Hernanz DF (2003) Patterns and relative rates of nucleotide and insertion/deletion evolution at six chloroplast intergenic regions in New World species of the Lecythidaceae. Mol Biol Evol 20:1710–1721

Hao B, Wang L, Mu L et al (2006) A study of conservation genetics in *Cupressus chengiana*, an endangered endemic of China, using ISSR markers. Biochem Genet 44:31–45

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc Lond B 359:183–195

Huang SSF, Hwang S-Y, Lin T-P (2002) Spatial pattern of chloroplast DNA variation of *Cyclobalanopsis glauca* in Taiwan and East Asia. Mol Ecol 11:2349–2358

Hwang S-Y, Lin HW, Kuo YS, Lin TP (2001) RAPD variation in relation to population differentiation of *Chamaecyparis formosensis* and *Chamaecyparis taiwanensis*. Bot Bull Acad Sin 42:173–179

Hwang S-Y, Lin TP, Ma C-S, Lin C-L (2003) Postglacial population growth of *Cunninghamia konishii* (Cupressaceae) inferred from phylogeographical and mismatch analysis of chloroplast DNA variation. Mol Ecol 12:2689–2695

Isabel N, Tremblay L, Michaud M et al (1993) RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill.) B.S.P. Theor Appl Genet 86:81–87

Jagel A, Stützel TH (2001) Untersuchungen zur Morphologie und Morphogenese der Samenzapfen von *Platycladus orientalis* (L.) Franco (=*Thuja orientalis* L.) und *Microbiota decussata* Kom. (Cupressaceae). Bot Jahrb Syst Pflanzengesch Pflanzengeogr 123:377–404

Jaramillo-Correa JP, Beaulieu J, Ledig FT, Bousquet J (2006) Decoupled mitochondrial and chloroplast DNA population structure reveals Holocene collapse and population isolation in a threatened Mexican-endemic conifer. Mol Ecol 15:2787–2800

Jump AS, Peñuelas J (2006) Genetic effects of habitat fragmentation in wind-pollinated tree. Proc Natl Acad Sci USA 103:8096–8100

Komarov VL (1923) On some new Asian gymnosperms. Botanicheskie Materialy Gerbariya Glavnogo Botanicheskogo Sada RSFSR 4(23/24):177–181

Kondo T, Tsumura Y, Kawahara T, Okamura M (1998) Paternal inheritance of chloroplast and mitochondrial DNA in interspecific hybrids of *Chamaecyparis* spp. Breed Sci 48:177–179

Koropachinskii IY (1989) Cupressaceae Bartl. In: Kharkevich SS (ed) The vascular plants of the Soviet Far East, vol 4. Nauka, Leningrad, pp 20–24

Krestov PV, Verkholat VP (2003) Rare plant communities of Amur region. Institute of Biology and Soil Sciences, Vladivostok

Kurentsova GE (1968) Relict plants of Primorye. Nauka, Leningrad

Little DP, Schwarzbach AE, Adams RP, Hsieh C-F (2004) The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). Am J Bot 91:1872–1881

Manchester SR, Chen Z-D, Lu A-M, Uemura K (2009) Eastern Asian endemic seed plant genera and their paleogeographic history throughout the northern hemisphere. J Syst Evol 47:1–42

Marshall HD, Newton C, Ritland K (2001) Sequence-repeat polymorphisms exhibit the signature of recombination in lodgepole pine chloroplast DNA. Mol Biol Evol 18:2136–2138

Melnikova AB, Machinov AN (2004) On the record of *Microbiota decussata* (Cupressaceae) at unusually low altitude. Bot Z 89:1470–14720

Moiseev PA, Shiyatov SG (2003) Vegetation dynamics at the treeline ecotone in the Ural highlands, Russia. In: Nagy L, Grabherr G, Körner C, Thompson DBA (eds) Alpine biodiversity in Europe—a Europe-wide assessment of biological richness and change. Ecological studies, vol 167. Springer, Berlin, pp 423–435

Nagiri Y, Gaudeul M (2007) Phylogeography of the endangered *Eryngium alpinum* L. (Apiaceae) in the European Alps. Mol Ecol 16:2721–2733

Neale DB, Marshall KA, Sederoff RR (1989) Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don Endl. Proc Natl Acad Sci USA 86:9347–9349

Ouborg NJ, Piquot Y, Van Groenendael JM (1999) Population genetics, molecular markers and the study of dispersal in plants. J Ecol 87:551–568

Panchal M (2007) The automation of nested clade phylogeographic analysis. Bioinformatics 23:509–510

Pavlyutkin BI, Petrenko TI, Chekryzhov IY (2005) The problems of stratigraphy of the Tertiary deposits of the Pavlovka coal-field (Primorye). Tikhookeanskaya Geologiya 24(6):59–76

Petit RJ (2008) The coup de grâce for the nested clade phylogeographic analysis? Mol Ecol 17:516–518

Petit RJ, Duminil J, Fineschi S et al (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Mol Ecol 14:689–701

Petit RJ, Hu FS, Dick CW (2008) Forests of the past: a window to future changes. Science 320(5882):1450–1452

Pimenov GM (1990) Miocene conifers of the south Far East and their stratigraphical significance. Far Eastern Branch of the Academy of Sciences of the USSR, Vladivostok

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237–1245

Posada D, Crandall KA, Templeton AR (2000) GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol Ecol 9:487–488

Provan J, Soranzo N, Wilson NJ et al (1998) Gene-pool variation in Caledonian and European Scots pine (*Pinus sylvestris* L.) revealed by chloroplast simple-sequence repeats. Proc R Soc Lond B 265:1697–1705

Provan J, Beatty GE, Hunter AM et al (2007) Restricted gene flow in fragmented populations of a wind-pollinated tree. Conserv Genet 9:1521–1532

Ramos-Onsins S, Rozas J (2002) Statistical properties of new neutrality tests against population growth. Mol Biol Evol 19(12):2092–2100

Red Data Book Primorsky Kray (2008) Plants. Rare and endangered species of plants and fungi. In: Kozhevnikov AE, Nedoluzhko VA, Barkalov VYu et al (eds) AVK "Apelsin", Vladivostok, pp 339–341

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237

Renau-Morata B, Nebauer SG, Sales E et al (2005) Genetic diversity and structure of natural and managed populations of *Cedrus atlantica* (Pinaceae) assessed using random amplified polymorphic DNA. Am J Bot 92:875–884

Rendell S, Ennos A (2003) Chloroplast DNA diversity of the dioecious European tree *Ilex aquifolium* L. (English holly). Mol Ecol 12:2681–2688

Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145:1219–1228

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Schaal BA, Gaskin JF, Caicedo AL (2003) Phylogeography, haplotype trees, and invasive plant species. J Hered 94(3):197–204

Shaw J, Lickey EB, Beck JT et al (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Bot 92:142–166

Shiraishi S, Maeda H, Toda T et al (2001) Incomplete paternal inheritance of chloroplast DNA recognized in *Chamaecyparis obtusa* using an intraspecific polymorphism of the trnD-trnY intergenic spacer region. Theor Appl Genet 102:935–941

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381

Soja AJ, Tchebakova NM, French NHF et al (2007) Climate-induced boreal forest change: predictions versus current observations. Global Planet Chang 56:274–296

Sosa V, Ruiz-Sanchez E, Rodriguez-Gomez FC (2009) Hidden phylogeographic complexity in the Sierra Madre Oriental: the case of the Mexican tulip poppy *Hunnemannia fumariifolia* (Papaveraceae). J Biogeogr 36:18–27

Stockey RA, Hill RS, Kvacek J (2005) Fossil record of Cupressaceae s. lat. In: Farjon A et al (eds) A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew, UK, pp 54–68

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Templeton AR (2008) Nested clade analysis: an extensively validated method for strong phylogeographic inference. Mol Ecol 17:1877–1880

Truong C, Palme AE, Felber F (2007) Recent invasion of the mountain birch *Betula pubescens* ssp. *tortuosa* above the treeline due to climate change: genetic and ecological study in northern Sweden. J Evol Biol 20:369–380

Urusov VM (1979) Ecological and biological peculiarities of *Microbiota decassata* Kom. (Cupressaceae). Bot Z 64:362–376

Urusov VM (1988) Genesis of vegetation and conservancy in the Far East. Far Eastern Branch of the Academy of Sciences of the USSR, Vladivostok

Volynets EB (2005) Aptian-Cenomanian flora of Primorye. Part 1. Floristic assemblages. Stratigr Geol Correl 13(6):613–631

Vyshin IB (1990) High-altitude vascular plants of Sikhote Alin. Far Eastern Branch of the Academy of Sciences of the USSR, Vladivostok

Walter R, Epperson BK (2005) Geographic pattern of genetic diversity in *Pinus resinosa*: contact zone between descendants of glacial refugia. Am J Bot 92:92–100

Walther GR, Beißner S, Pott R (2005) Climate change and high mountain vegetation shifts. In: Broll G, Keplin B (eds) Mountain ecosystems: studies in treeline ecology. Springer, Berlin, pp 77–96

Zamyatnin BN (1963) On the specification of characteristics of *Microbiota decussata* Kom. Botanicheskie Materialy Gerbariya Botanicheskogo Instituta im Komarova AN SSSR 22:43–50

Zhang Q, Chiang TY, George M et al (2005) Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. Mol Ecol 14:3513–3524