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ORIGINAL PAPER

Population genetic structure, local adaptation, and conservation genetics of *Kandelia obovata*

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Abstract Topographic changes during the Pleistocene glacial/interglacial cycles affected the distribution of coastline mangroves and influenced their population genetic structure. The submergence of the continental shelf off southeast China during the postglacial age caused coastline expansions and resulted in the colonization of mangroves. Here, we performed multilocus genome scans using amplified fragment-length polymorphisms to explore the effects of topography and natural selection in structuring *Kandelia obovata* populations. Long-term isolation by the Taiwan Strait since the end of the last glacial maximum, which obstructed gene flow, differentiated the Taiwanese and Chinese populations. Founders that colonized from both outlets of the Taiwan Strait were sourced from the northern South China Sea and the Ryukyus, thereby creating a melting pot in the Taiwan Strait. Inner-strait currents played roles as vectors for propagule dispersal among populations. Upon examination of the allele-frequency distributions of outlier loci, most negative outliers reflected the widespread polymorphisms shared by common ancestors. Furthermore, significant differentiation in the genetic components of positive outliers between this and other populations and the negative correlation with geographic distance suggested the presence of geography- or latitude-independent population divergence. Restored populations were compared with their sources and revealed biased sampling of nursery seedlings, which caused within-population substructures and reduced effective population sizes. This study indicated that multiple factors affect the population structure of the mangroves off southeast China.

Keywords Population structure · AFLP · Topographic effect · Demographic effect · *Kandelia obovata*

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Introduction

Geography significantly affects the population genetic structure of mangrove species (Duke et al. 2002; Thiel and Haye 2006; Triest 2008). Land barriers for species distribution or gene flow have been studied in several mangrove species, e.g., the genetic differentiation between the mangrove populations of the South China Sea and the Bay of Bengal due to isolation by the Malay Peninsula (Huang et al. 2008; Liao et al. 2007, 2009; Su et al. 2006). Seawaters play the roles of barriers to gene flow (Deng et al. 2009; Duke et al. 2002) as well as vectors for propagule dispersal (Castillo-Cardenas et al. 2005; Liao et al. 2009). These conflicting functions of seawaters are resolved by the geographic distance, ocean currents, and submarine topography (Duke et al. 2002; Triest 2008). Changes in the coastline due to interglacial sea-level rise and glacial surface subsidence affect mangrove distributions and

result in tight connections between geohistory and the population genetic structure of mangroves (Liao et al. 2007, 2011). In addition to geographic effects, selective pressures such as sediment characteristics, metal levels, saline levels, temperatures, water-logging time, etc. may also affect the genetic structure (Afzal-Rafii et al. 1999; Melville and Burchett 2002), growing pattern, and species distribution (Chen et al. 2004; Kitaya et al. 2002), and may even promote speciation in mangroves (Chen et al. 2011; Zhou et al. 2011). These studies have indicated that the genetic structure of mangroves might be affected by multiple factors, including geographic barriers, demographic history, and intrinsic adaptability.

Water currents and geographic distance play important roles in structuring the population genetics of species of *Kandelia* (Chiang et al. 2001; Giang et al. 2006). Genetically differentiated populations between the northern and southern populations of the South China Sea have been recognized as different species based on micro- and macro-morphological evidence (Sheue et al. 2003), although several studies still use the misidentified name *K. candel* (L.) Druce for the northern species (e.g., Chen et al. 2004; Geng et al. 2008; Ye et al. 2005, 2010). The northern species, *Kandelia obovata* Sheue, Liu & Yong, is distributed in the subtropical Pacific and around the northern margins of the mangroves in the Indo-West Pacific. Genetic isolation between the populations of Chinese *K. obovata* has been evidenced by intersimple sequence repeat markers and has been suggested to be a result of geographic isolation and the drift effect (Chen et al. 2010). Small-scale spatial analysis indicated that limited propagule dispersal distances and the mating system would result in a half-sib family structure (Geng et al. 2008). Temperature, especially winter temperature, is probably the key factor affecting the habitat of *K. obovata* (Analuddin et al. 2009; Gwada et al. 2000; Hogue et al. 2010). Although the Kuroshio Current carries warm waters from the tropic and raises the temperature of *K. obovata* habitats, this species is believed to be adapted to a cooler climate than are other mangrove species (Kao et al. 2004).

The Taiwan Strait is around 70–90 m in depth and has a bulge in the central part, forming the Penghu archipelago. The topography of the shallow shelf of the Taiwan Strait is caused by the uplift of the Taiwan orogen, both by oblique collision between the Luzon Arc and the Chinese margin since the Pliocene period and by foreland-basin sedimentation (Yu and Song 1993; Yu 2003; Yu and Chou 2001). This shallow strait emerged and connected Taiwan Island and mainland China during the Pleistocene glacial period (Lin 1963; Yu 2003), resulting in the retreat and disappearance of the coastlines of the western and eastern Taiwan Strait. In other words, the mangroves distributed around the Taiwan Strait were not in existence then, and the present populations might be descendents of founders that arrived since the last interglacial period. In addition, the colonization and dispersal of mangroves were probably influenced by seasonally varied currents in the Taiwan Strait; the southward China Coastal Current (CCC) dominates the winter currents of the strait but gradually weakens in the spring, and the northward South China Sea Current (SCSC) and the Kuroshio Branch Current (KBC) begins its advance in the spring, dominates in the summer, and weakens in the autumn (Liao et al. 2008). The major northward SCSC and KBC and the minor southward CCC in the spring might guide the dispersal directions of *K. obovata* propagules, which mature and germinate between March and May.

For conservation purposes, the reforestation of mangroves from nursery seedlings is commonly used to increase the census population size. Transplantation of seedlings to new habitats could cause the founder effect, which results in lower genetic diversity. Microclimate variations in habitats could also result in the loss of locally adapted genes (McKay et al. 2001) but have less influence on overall divergent loci (Barton and Bengtsson 1986). Local governments in China have reforested mangroves, such as *K. obovata*, from southern populations (Fujian Province) to higher latitudes (Zhejiang Province) for decades. Although the natural population is threatened due to harbor extension and environmental pollution, successful reforestation has increased the census population size of *K. obovata* in China. However, the change in the genetic diversity of *ex situ* restored populations is unknown, as is the influence of the founder effect and of microclimates that are different from that of the source.

For clarifying the demographic and adaptive effect on the population genetic structure, we adopt the multilocus genome scan approach to investigate the genome-wide genetic diversity of *K. obovata* along the coasts of the Taiwan Strait. The genome scan analysis is a kind of method to detect the outlier loci that show unexpected high or low genetic differentiation, which are putatively under selection or link with the adaptive genes (Beaumont and Balding 2004; Beaumont and Nichols 1996). Based on such method, the putatively neutral loci and the outlier loci can be separately used to estimate the genetic diversity and genetic structure among populations. Although the high amount of false positive could cause overestimates of the number of putatively adaptive genes under the island model with spatial migration (Excoffier et al. 2009; Meirmans 2012), comparisons of genetic distribution between the neutral and outlier loci can still provide clues to resolve the relation between population structure and geographic distribution.

In this study, the multilocus genome scan approach was adopted to explore the adaptive effects and demographic effect on the genetic differentiation of *K. obovata* in China and Taiwan, based on separate analyses of neutral loci and candidate selective loci. The degrees of genetic diversity and genetic differentiation between natural populations, and between restored and corresponding source populations,

were examined to explore the following issues: (1) topographic and geohistorical effects on the population structure of *K. obovata*, (2) demographic and local adaptation effects on the genetic diversity and population structure of *K. obovata*, and (3) differences in genetic variation between restored populations and the corresponding source population. This study should provide clues towards understanding the sources of genetic diversity and the causes for the population structure of extant *K. obovata* along the Taiwan Strait. Comparison of genetic diversity between restored populations and natural populations also provides evidence of the founder effect by sampling bias, which is an important issue for conservation management.

Materials and methods

Sampling

K. obovata is distributed along the coasts of southeast China, Taiwan, and the Ryukyus archipelagos, and is latitudinally isolated from its sister species, *K. candel*, with respect to genetics (Chiang et al. 2001; Giang et al. 2006) and morphology (Giang et al. 2006; Sheue et al. 2003). In this study, the effect of the topographic isolation of natural populations by the Taiwan Strait was investigated. Therefore, the sampling was focused on populations on Taiwan Island and in southeast China. Leaf samples of *K. obovata* were collected from the natural populations in FuDing (FD), ZhangJiang Estuary (ZJ), and ShenZhen (SZ) on the southeastern coast of China, DongZhaiGang (DZG) in North Hainan Island, and JuWei (JW) in North Taiwan Island (Fig. 1). Comprehensive sampling was implemented along the riversides where *K. obovata* grows. Only adult individuals with diameter at breast height over 5 cm and at minimum distance of 5 m from each other were collected in preventing the sampling bias (identical by descent). In addition, we explored changes in the genetic diversity of restored populations compared to the natural populations; for this, the restored populations at LongGang (LG) and YueQing Bay (YQ) were sampled for comparison with the corresponding source populations. According to the official records of the local managers of the restored populations, the population LG was sourced from seedlings of FD collected in 1999 and 2008, and coded as LG1 and LG2, respectively. The history of the restoration of YQ is longer (since 1957), but the exact source is unclear and could only be traced to the coasts of the Fujian Province of China. In total, 154 and 72 individuals were collected from natural and restored populations, respectively (Table 1). Detailed geographic information is listed in Table 1. Weather conditions of local populations are provided in Table S1 (Online Resource).

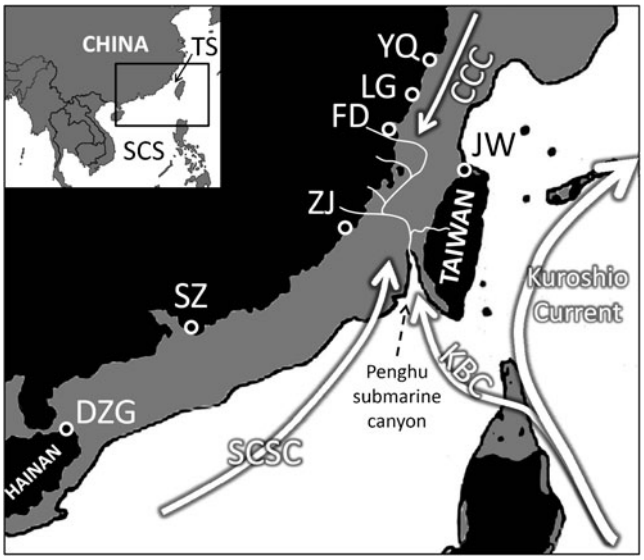

Fig. 1 Map of sampling areas in this study. The *gray area* is the continental shelf that emerged during the Pleistocene glacial period. The *black arrow* indicates the Penghu submarine canyon, the ancient estuary of Minchiang (Min River, indicated by the *white lines*) sourced from both China and Taiwan (Huang and Yu 2003); *white arrows* indicate the directions of sea currents. *YQ*, *LG*, *FD*, *ZJ*, *SZ*, *DZG*, and *JW* are abbreviations of sampling locations as indicated in Table 1; *TS* and *SCS* in the *top left panel* indicate the Taiwan Strait and the South China Sea, respectively; *CCC*, *SCSC*, and *KBC* indicate the China Coast Current, South China Sea Current, and Kuroshio Branch Current, respectively

DNA extractions and AFLP genotyping

Fresh leaves were dried using silica gel and ground to powder by liquid nitrogen immediately after transfer to the laboratory. Total genomic DNA was extracted using the cetyl trimethylammonium bromide method (Doyle and Doyle 1987). The extracted DNA was dissolved in 1× TE buffer and stored at −20 °C.

For the multilocus genome scan approach, we adopted the amplified fragment length polymorphism (AFLP) technique for genetic assessment. AFLP was performed following the method developed by Vos et al. (1995) with little modification. A total of 250 ng DNA was digested by the restriction enzymes *Eco*RI (10 U) and *Mse*I (10 U) (New England Biolabs, Beverly, MA, USA) in a total reaction volume of 25 μL in 37 °C for 3 h, followed by incubation at 70 °C for 15 min to inactivate the enzymes. The digested products (5 μL) were added to 15 μL ligation mix with 5 pmol *Eco*RI adapter, 50 pmol *Mse*I adapter, and 1 U T4 DNA ligase (New England Biolabs, Beverly, MA, USA) and incubated at 16 °C for 1 h, followed by 37 °C for 3 h. The ligated products were pre-selected using 0.5 mM of the primers Eco+A (GACTGCGTACCAATTCA) and Mse+C (GATGAGTCCTGAGTAAC), with 2.5 nmol dNTP, 3 nmol $MgCl_2$, 0.2 μL 1 % BSA, and 1 U DNA Taq polymerase. Amplification reactions were performed at 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min with 20 cycles. Pre-

Table 1 Information regarding the sampling areas in this study

Population	Code	Sample size	Geographic area	Latitude	Longitude	Restoring year	Source
Restored population							
LongGang	LG1	20	Southeast China	27°35′	120°34′	1999	FD
LongGang	LG2	29	Southeast China	27°35′	120°34′	2008	FD
YueQing Bay	YQ	23	Southeast China	28°25′	120°56′	1957	Coasts of Fujian Province
Natural population							
FuDing	FD	41	Southeast China	26°52′	119°55′		
ZhangJiang Estuary	ZJ	29	Southeast China	23°55′	117°24′		
ShenZhen	SZ	19	South China	22°32′	114°03′		
DongZhaiGang	DZG	30	Hainan Island	19°57′	110°35′		
JuWei	JW	35	Taiwan Island	25°08′	121°27′		

selected products were used as templates for selective amplification. Selective amplifications were conducted using 0.5 mM of the primer pairs Eco+AGT/Mse+CTA, Eco+ACG/Mse+CTC, and Eco+AAT/Mse+CTG. These primers were labeled with fluorescence dyes (6FAM, JOE, and TAMRA, respectively), with 3 nmol $MgCl_2$, 2.5 nmol dNTP, and 1 U DNA Taq polymerase. Selective PCR was performed using the following temperature settings: 94 °C for 2 min for reaction activation, followed by a total of 25 cycles of 94 °C for 30 s, 65 °C for 30 s (with a decrease 1 °C every cycle until it reached 56 °C), and 72 °C for 1 min, with a further 30 min at 72 °C for the final extension. Low-numbered PCR cycles were adopted for preselected and selective amplification (20 and 25 cycles, respectively) for reducing the PCR error. The concentrations of the selected amplified products were checked using 1.5 % agarose gels. Genotyping was performed on an ABI Prism 3730XL instrument (Applied Biosystems, Foster City, CA, USA). LIZ600 was used as the size standard, and peak size detection was conducted using Peak Scanner software ver. 1.0 (Applied Biosystems). We repeated the experiments several times to ensure the band (peak) signals in the preliminary tests for affirming the presence of bands (peaks) is not caused of PCR error. Negative control using the water as the template was used for excluding ghost bands. Only the loci that were consistently present and absent in all preliminary tests were read in the formal experiment.

Data scoring and data analyses

AFLP bands with the same migration distances were considered homologous loci and scored manually as present (1) or absent (0). The sizes of the AFLP bands scored ranged from 50 to 300 bps. In order to reduce the bias for band coding, the 0/1 coding is double checked by at least two persons. We used the method of Beaumont and Nichols (1996) to identify the F_{ST} outlier for each locus individually. The idea of outlier loci is based on the hypothesis that extremely high F_{ST} between populations are seen in positively selected loci (the positive outliers) compared to neutral loci, and reduced F_{ST} in loci under balancing selection (the negative outliers). The program Mcheza (Antao and Beaumont 2011) was used to detect the outlier F_{ST} loci by two strategies: (1) we ran Mcheza once to calculate the mean F_{ST} from empirical data, followed by forcing the simulation according to the mean F_{ST}; (2) we ran Mcheza as in strategy 1 but ran the simulation after removing the loci outside the 95 % confidence interval as recommended by Antao et al. (2008). One million Markov chain simulations were performed. Each strategy was run three times to obtain a converged inference to ensure the accuracy of estimation. The dataset was further divided into neutral loci and positively selected loci, in order to execute the following analyses separately.

Genetic diversity indices, including the percentage of polymorphic loci, number of effective alleles (A), unbiased heterozygosity (H_e), and Shannon's information index (I) of each population were further estimated using the neutral loci by GenAlEx 6.3 (Peakall and Smouse 2006). Genetic diversity between the natural populations and the restored populations were compared using Wilcoxon two-sample test using JMP6.0 (SAS Institute Inc., Cary, NC, USA). Effective population size (N_e) was estimated for each population using neutral loci based on Jorde et al.'s (1999) method for dominant gene marker frequencies. Because the allele frequency of two generations is wanting in natural populations, we used the total recessive allele frequency of natural populations in replace of the recessive allele frequency of parental population (q_x, Eq. 6 of Jorde et al. (1999)). Therefore, the estimated N_e of each population is indicative of relative population size in comparison with total populations. The N_e is also estimated for the restored populations using the recessive allele frequency of the source

population as the q_x. Detailed parameters used for estimating the N_e are listed in Table S2 (Online Resource).

The distribution of the genetic diversity of neutral loci among natural populations between the western Taiwan Strait and the eastern Taiwan Strait was evaluated by analysis of molecular variance (AMOVA). Principle coordinate analysis (PCoA) was also performed to evaluate the genetic composition among natural populations. Both AMOVA and PCoA were reanalyzed using the positive outlier loci in comparison with the neutral loci. The PCoA were also performed to evaluate the genetic homogeneity between the restored populations LG1 and LG2 and the corresponding source population FD. The assignment test based on Bayesian clustering analysis was performed to examine both the genetic structure among populations and the degrees of genetic admixture among populations, using STRUCTURE ver. 2.3.3 (Falush et al. 2003, 2007; Pritchard et al. 2000). The admixture model was used (Hubisz et al. 2009). The posterior probability of the grouping number (K=1–10) was estimated by the Markov chain Monte Carlo method with 10 independent runs to evaluate the consistency of the results, using 3,000,000 steps with a 500,000-step burn-in for each run. The best and the better grouping numbers were evaluated using ΔK (Evanno et al. 2005) by STRUCTURE HARVESTER v. 0.6.8 (Earl and vonHoldt 2012). A final 10,000,000 simulations with a 1,000,000-step burn-in was executed based on the best K.

Results

Neutrality test and AFLP polymorphisms

In total, 221 polymorphic loci were obtained from 204 samples (including natural and restored populations) based on three AFLP RE-adaptor/primer sets. Twelve (5.43 %) and 30 (13.57 %) of the 221 loci had significantly higher and lower F_{ST} values deviating from 95 % confidence intervals, respectively, and were defined as positive and negative outliers, respectively (Fig. S1, Online Resource).

Genetic diversity of populations

The AFLP markers revealed 93.85 and 91.62 % polymorphic-neutral loci in natural and restored populations, respectively. In the natural populations, the number of effective loci (A) of all populations was 1.641±0.023, ranging from 1.521±0.027 (SZ) to 1.659±0.025 (DZG); the Shannon's index (I) was 0.532±0.015, ranging from 0.449±0.018 (SZ) to 0.527±0.016 (FD); the expected heterozygosity (H_e) was 0.366±0.011, ranging from 0.318±0.014 (SZ) to 0.357±0.013 (FD). Generally, the FD population had the highest diversity compared to the others (P<0.05, Student's t test, Table 2), while SZ had relatively lower diversity. Among the three restored populations examined, LG1 had significantly higher genetic diversity in A (1.610±0.026), I (0.500±0.017), and H_e (0.362±0.014) than did LG2 and YQ (Table 2). The genetic diversity of the sampling populations was higher than the general estimates of central populations (H_e≈0.2) using the AFLP marker (Triest 2008). The N_e estimated by the neutral loci revealed relatively small effective population sizes of restored populations (N_e=21.321) than the natural populations (N_e=30.591) (Table 2 and Table S2, Online Resource). The most southern population DZG had the highest N_e (N_e=22.477), and the SZ had the lowest N_e (N_e=11.637) among natural populations. Restored populations LG1 (N_e=7.749) and LG2 (N_e=10.038) had relatively small N_e than the source population FD (N_e=17.362); the decrease in N_e was also inferred in another restored population

Table 2 Summary of genetic diversity of natural and restored populations estimated by neutral loci and positive outlier loci

Population	Neutral loci					Positive outliers			
	PPL (%)	A	I	H_e	N_e	PPL (%)	A	I	H_e
Restored pops	91.62	1.579±0.025	0.495±0.016	0.339±0.012	21.321	100	1.557±0.077	0.516±0.039	0.345±0.034
LG1	87.15	1.610±0.026^{ab}	0.500±0.017^{ab}	0.362±0.014^{ab}	7.749	91.67	1.487±0.098^{ab}	0.451±0.061^{ab}	0.312±0.049^{ab}
LG2	84.92	1.488±0.026^{d}	0.436±0.018^{d}	0.300±0.013^{c}	10.038	100	1.309±0.065^{bc}	0.361±0.046^{ab}	0.225±0.037^{ab}
YQ	86.03	1.485±0.025^{d}	0.436±0.017^{d}	0.302±0.013^{c}	11.430	75.00	1.363±0.112^{abc}	0.331±0.079^{ab}	0.225±0.060^{ab}
Natural pops	93.85	1.641±0.023	0.532±0.015	0.366±0.011	30.591	100	1.745±0.085	0.853±0.052	0.409±0.040
FD	91.06	1.656±0.026^{a}	0.527±0.016^{a}	0.375±0.013^{a}	17.362	91.67	1.540±0.101^{ab}	0.475±0.063^{a}	0.327±0.050^{a}
ZJ	90.50	1.532±0.024^{cd}	0.472±0.016^{bcd}	0.326±0.012^{c}	16.286	91.67	1.484±0.100^{abc}	0.445±0.064^{ab}	0.302±0.050^{ab}
SZ	84.92	1.521±0.027^{cd}	0.449±0.018^{cd}	0.318±0.014^{c}	11.637	91.67	1.229±0.056^{c}	0.295±0.048^{b}	0.179±0.036^{b}
DZG	90.50	1.569±0.025^{bc}	0.487±0.016^{abc}	0.336±0.013^{bc}	22.477	83.33	1.607±0.093^{a}	0.504±0.071^{a}	0.355±0.051^{a}
JW	89.94	1.526±0.024^{cd}	0.469±0.016^{bcd}	0.329±0.013^{bc}	12.106	91.67	1.387±0.092^{abc}	0.391±0.059^{ab}	0.260±0.048^{ab}

Levels not connected by the same letter are significantly different based on the Student's t test

PPL percentage of polymorphic loci, A number of effective loci, I Shannon's information index, H_e unbiased heterozygosity, N_e effective population size estimated by the equations of Jorde et al. (1999)

YQ, which had relatively small N_e (N_e=11.430) than any examined natural populations in the Fujian Province of China (Table 2 and Table S2, Online Resource).

In addition to the neutral loci, genetic diversity was also estimated using the positive outliers to examine the effects of local adaptation in natural populations and sampling bias in nursery seedlings. The percentages of polymorphic outlier loci in both natural and restored populations were 100 %. The overall A, I, and H_e of the natural populations were 1.745±0.085, 0.853±0.052, and 0.409±0.040, respectively. In general, SZ had the lowest diversity (A=1.229±0.056, I=0.295±0.048, and H_e=0.179±0.036), but was not significantly different from ZJ and JW, and DZG had the highest (A=1.607±0.093, I=0.504±0.071, and H_e=0.355±0.051), but was not significantly different from other populations except SZ. In the restored populations, the genetic diversities estimated by positive outliers were not significantly different from each other (Table 2).

Genetic structure among natural populations

The population genetic structure inferred by neutral loci revealed that most variations were observed within populations (86 %), and only an 8 % variation differentiated the populations of Taiwan and China. Distributions of variation among regions, among populations within regions, and within populations inferred by Φ statistic were 0.084, 0.064, and 0.143, respectively (P=0.001 at all hierarchies, Table 3). In contrast to the significant genetic differentiation inferred by neutral loci, no genetic differentiation was detected in the positive outliers between the eastern and the western coasts of the Taiwan Strait (Φ_{CT}=−0.048, P=1.000), indicating that the population differentiation between regions could be significantly affected by geographic isolation instead of local adaptation (or natural selection). Relatively high genetic differentiation was detected among populations within regions (Φ_{SC}=0.324, P=0.001) and within populations (Φ_{ST}=0.291, P=0.001) compared to the neutral loci estimates, revealing that the local adaptations could have occurred in small geographic ranges.

Pairwise genetic distance was also calculated using the pairwise Φ statistic and revealed significant genetic differentiation between most population pairs, except between DZG and ZJ in either neutral loci (Φ_{ST}=0.011, P=0.055) or positive outlier loci (Φ_{ST}=0.011, P=0.202) (Table 4). In the estimates of neutral loci, the genetic distance between the Taiwanese population (JW) and the Chinese populations was relatively higher than the genetic distance between populations within China. Unsurprisingly, the Φ_{ST} of the positive outliers was higher than that of the neutral loci, but the genetic distance between the Taiwanese and Chinese populations ranged from 0.152 to 0.491, which is similar to the genetic distance between populations within China (Φ_{ST}=0.011–0.506, Table 4). This result is consistent with the result of significant population structure estimated using AMOVA.

Genetic homogeneity among natural populations

Homogeneity of genetic composition among populations was determined by several methods, including the neighbor-joining (NJ) tree method, PCoA, and Bayesian clustering analysis by STRUCTURE ver. 2.3.3 (Falush et al. 2003, 2007; Pritchard et al. 2000). The NJ tree reconstructed from neutral loci showed an admixture pattern of populations in the west Taiwan Strait (China) with a clearly distinct clade of JW in Taiwan (Fig. 2a). Although JW has a distinct relationship from the others, three samples were assigned into clades of Chinese populations, implying genetic immigrations. However, Bayesian clustering analysis revealed different grouping patterns, in that FD and JW had genetic components similar to each other but different from the others when assigning two groups (K=2), which was the best grouping manner by the ΔK evaluation (ΔK=772.76 when K=2, Fig. 3). If K=3, which has the second high ΔK value (ΔK=346.57), was considered, JW was separated from other Chinese populations; ZJ, SZ, and DZG shared certain genetic

Table 3 Analysis of molecular variance (AMOVA) of natural populations by neutral loci and positive outliers

Source	*df*	S.S.	Var. comp.	% variation	Φ	*P* value
Neutral loci						
Among regions	1	171.114	2.994	8	0.084	0.001
Among populations	3	268.548	2.081	6	0.064	0.001
Within populations	130	3947.213	30.363	86	0.143	0.001
Total	134	4386.874	35.439	100		
Positive outliers						
Among regions	1	15.613	−0.123	0	−0.048	1.000
Among populations	3	78.994	0.863	32	0.324	0.001
Within populations	130	234.430	1.803	68	0.291	0.001
Total	134	329.037	2.666	100		

The two regions were defined as the eastern coast (JW of Taiwan) and western coast (FD, ZJ, SZ, and DZG of China) of the Taiwan Strait

Table 4 Patterns of genetic differentiation revealed by pairwise Φ_{ST} by neutral loci and positive outliers between natural populations

	FD	ZJ	SZ	DZG	JW
FD	–	0.351**	0.457**	0.249**	0.422**
ZJ	0.103**	–	0.506**	0.011	0.154**
SZ	0.103**	0.040**	–	0.422**	0.491**
DZG	0.079**	0.011	0.044*	–	0.152*
JW	0.165**	0.132**	0.145**	0.135**	–

Above the diagonal: positive-outlier loci; below the diagonal: neutral loci

*P<0.005; **P<0.001; the other pairs were not significantly different (P>0.05)

composition traits with FD and revealed relatively closer genetic relationships with FD than with JW (Fig. 3). A slight increase in ΔK was shown in K=7 (ΔK=148.21), which shows admixture pattern of ZJ, SZ, and DZG, but the FD and the JW still well distinguished with small degrees of shared genetic components (Fig. 3). These results implied similar genetic compositions of the northern populations of China and Taiwan, but indicated that the long-term isolation by the Taiwan Strait has resulted in genetic differentiation between the populations in Taiwan and China.

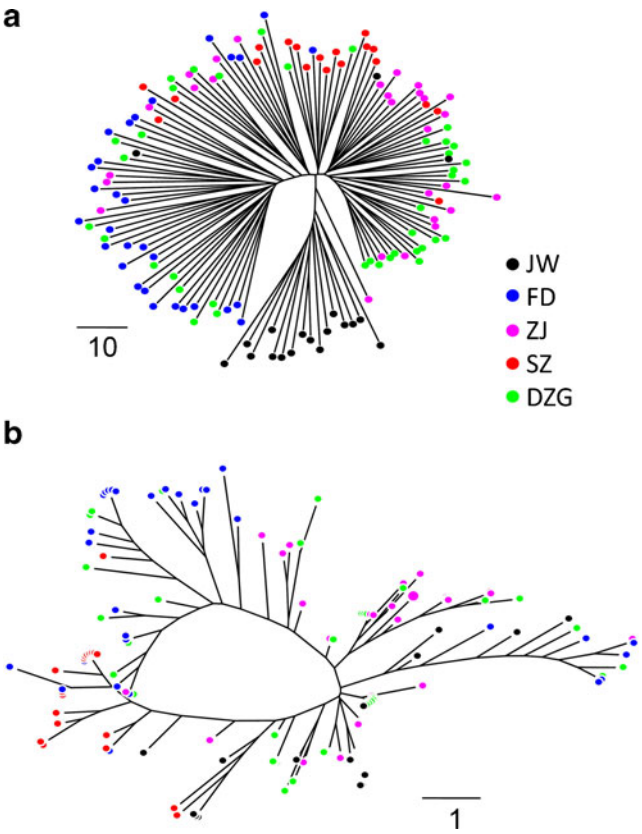

Fig. 2 Neighbor joining trees reconstructed by Nei and Li's (1979) genetic distance, generated from neutral loci (**a**) and positive-outlier loci (**b**), respectively

The ΔK is small at K=5 (ΔK=0.37) and the grouping is not corresponding to the five natural populations, which is similar to the results of K=3 and K=7, indicating the genetic admixtures among populations, especially for the ZJ, SZ, and DZG (Fig. 3).

In addition, the NJ tree was also reconstructed using positive outlier loci. Because the positive outliers were identified as candidate loci affected by positive selection (Beaumont and Balding 2004), the tree topology reflected the adaptation pattern. If the grouping pattern is clearly consistent with geographical distribution in positive outliers rather than in neutral loci, local adaption can be taken as the major force in population differentiation; in contrast, if the grouping of populations reveals a mosaic pattern, the selective forces cannot be identified, i.e., they may be shared by different populations or common ancestors. In our result, irregular grouping was seen in the tree of positive outliers, even between the Taiwanese population (JW) and the Chinese populations, indicating that these populations might be not locally adapted by specific selective forces (Fig. 2b). Population genetic homogeneity was also examined by PCoA. The Taiwanese population (JW) was not distinct from Chinese populations at the first axis of PCoA in neutral loci, but was well separated from others at the second axis (Fig. 4a). Among the Chinese populations, FD and SZ could be roughly separated in the first axis, but the DZG and ZJ had wider distributions covering most Chinese populations. The PCoA of the positive outliers revealed distinct distributions between FD, ZJ, and SZ, while the genetic distribution of JW in Taiwan overlapped with that of ZJ (Fig. 4b). The southeast population DZG had wider distributions covering most populations except SZ, which was distinct from other populations at the second axis of the positive outlier PCoA. The PCoA results of neutral loci and positive outliers revealed that the Taiwanese population was similar to the Chinese populations in major genetic distribution, but showed partial differentiation from them.

Genetic comparisons between restored populations and the corresponding source population

Because the two areas (LG and YQ) used for restoring *K. obovata* have higher latitudes, they may experience relatively harsh conditions for survival (e.g., lower temperature). Therefore, we examined whether the genetic diversity of these restored populations was driven by natural selection. The genetic composition of neutral loci and positive outliers between LG1, LG2, and the corresponding source population FD were compared. If the genetic changes in progenies compared to the source population were due to natural selection, we expected similar genetic distributions of positive outliers in LG1

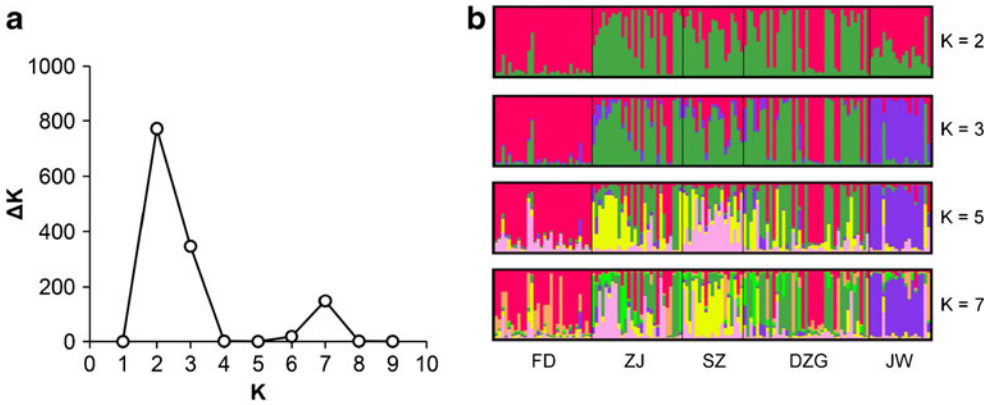

Fig. 3 Results of the Bayesian clustering analysis of natural populations inferred by neutral loci. **a** The best grouping number was 2 (K=2) based on the ΔK estimation (Evanno et al. 2005). **b** frequencies of genetic components of each individual separated by K=2, 3, 5, and 7

and LG2; in contrast, if the positive outlier distributions of LG1 and LG2 were different, the genetic change would be considered a consequence of sampling bias of nursery seedlings from the source population (FD).

The results of PCoA in neutral loci showed similar distributions in FD and LG1, distinct from LG2, in the first axis (Fig. 4c). Only small fraction of the FD samples incorporated with LG2, and the significant lower genetic diversity of LG2 (Table 2) indicated the founder effect in LG2 from the biased sampling of FD seedlings. A similar distribution pattern was also seen in the analysis of positive outliers (Fig. 4d). The distinct distributions of LG1 and LG2 in the positive outlier PCoA indicated that these two restored populations from the same area did not suffer identical selective forces but might have been artificially selected by sampling bias. This speculation was also supported by the highly significant correlation of heterozygosity between FD and LG1 in positive outlier loci (Table S3, Online Resource).

Discussion

Geohistorical topographies and ocean currents affecting the present population structure

Population genetic and phylogeographic studies focused on mangrove forests have shown that physical isolation (i.e., by land or water) plays an important role in predominating population structures (Dodd et al. 2002; Duke et al. 1998; Saenger 1998; Triest 2008). The genetic structure of the genus *Kandelia* was discovered using plastid DNA and revealed a distinct pattern between the north and the south populations in Southeast Asia (Chiang et al. 2001). Chiang et al. (2001) declared that currents might direct the dispersal routes of propagules of *Kandelia* and affect its genetic structure. Although the south and north populations were further evidenced as different species (i.e., *K. candel* in the south and *K. obovata* in the north), the effect of current directions on local population structures in mangroves should not be ignored.

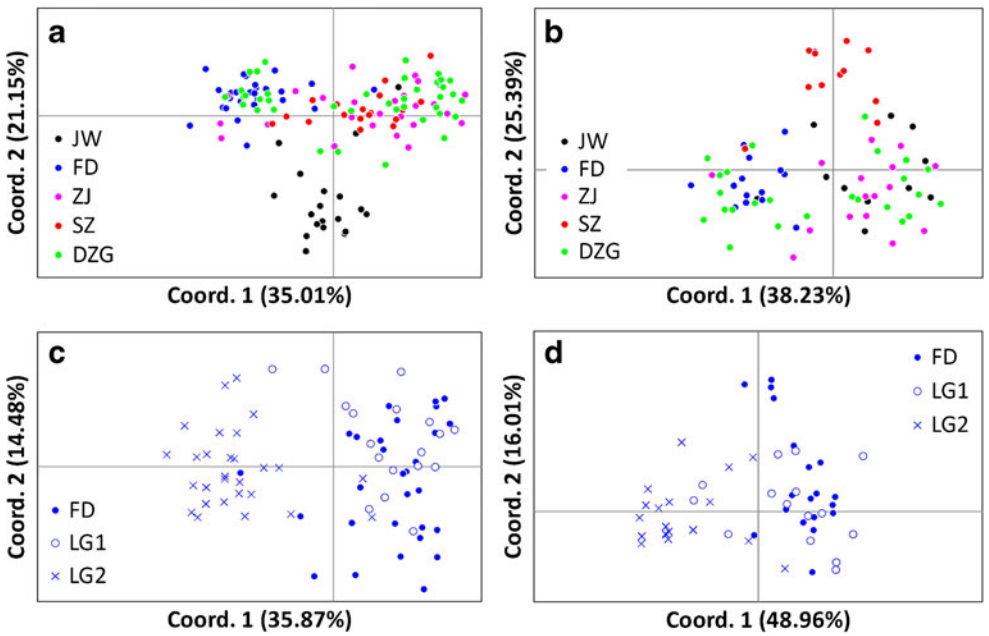

Fig. 4 Distribution of the first and the second axes of principle coordinate analysis (PCoA) for natural populations (**a**, **b**), and the restored populations LG1 and LG2 along with the corresponding source population FD (**c**, **d**), based on 179 neutral loci (**a**, **c**) and 12 positive outliers (**b**, **d**)

In this study, genetic differentiation was detected between the east and the west coasts of the Taiwan Strait, obviously reflecting the effect of the sea barrier. The geographic distance between FD and JW is roughly 245 km and is shorter than any other distance between natural populations in China (334–1,225 km), indicating the lack of a geographic-genetic association, which was confirmed by the lack of significant correlations between genetic and geographic distances by the Mantel test in neutral loci (Fig. S2, Online Resource). In addition, Bayesian clustering analysis showed that the best two clusters were the North group (FD and JW) and the South group (ZJ, SZ, and DZG; Fig. 3), which might imply different sources for the South and North populations. DZG has a broad genetic composition covering the other populations in the NJ tree (Fig. 2) and in the first axis in PCoA (Fig. 4), and reveals mosaic genetic components in Bayesian clustering analysis whenever K=3, 5, and 7 (Fig. 3); this population might have retained more ancestral polymorphisms and could have been a refugium during the last glacial maximum (LGM). Because the colonizing population was composed of descendents of founders from the origin center, the genetic components of colonizing populations (e.g., SZ and ZJ) are branches of the origin center (i.e., DZG). Geohistorical topography is the main factor affecting the genetic structure of mangrove species (Duke et al. 1998; Liao et al. 2011). The coastline during the LGM was different from that seen at present because of the emergence of the continental shelf (Fig. 1), which implies that the extant populations of coastal *K. obovata* were colonized from ancestors of ancient coastal populations after the LGM. The most probable dispersal route of *K. obovata* begins at the Penghu submarine canyon, colonizing northward following the retreat of the land and accelerated by the northward SCSC and KBC. Certain founders might have dispersed through the ancient watercourse of Minchiang (Min River), finally arriving at the northern locations (e.g., FD, Fig. 1). The northern South China Sea has a deeper seabed and formed the main part of the ancient South China Sea, which might have been one of the refugia of the West Pacific mangroves. Hainan Island, where DZG is located, was also thought to be the original center of *Ceriops tagal* of the South China Sea (Liao et al. 2011) despite the opposite colonization direction (i.e., southward) in *C. tagal*.

The second origin of *K. obovata* was probably from the northern islands, i.e., the Ryukyus. The North group (FD and JW) is composed of relatively simpler genetic components than the South group, as revealed in both STRUCTURE (Fig. 3) and PCoA (Fig. 4) analyses. The expansion of coastlines by the submergence of the deeper continental shelf off northern Taiwan likely caused a westward expansion of populations from the Ryukyus. The earlier submergence of the continental shelf off northern Taiwan (Fig. 1) likely provided opportunities for founders to colonize the coasts of the northern Taiwan Strait, including North Taiwan and East China. This would explain the similar genetic components of FD and JW in Bayesian clustering analysis at the best grouping number (K= 2, Fig. 3). The relatively high genetic diversity of FD and JW is probably consequences of multiple sources of colonizers from the Ryukyus and from the southern populations, although this speculation is not inconclusive and still needs more evidences (Table 2). In fact, JW is genetically differentiated from FD despite grouping with FD. This reflects the geographical effect of the barrier of the Taiwan Strait on the population structure of *K. obovata*.

Although different sources and the marine barrier have structured the extant populations of *K. obovata* (Tables 3 and 4), the relatively low Φ_{ST} suggests considerable gene flow among populations. The mating system of geitonogamous selfing (Sun et al. 1998) or neighboring outcrossing (Geng et al. 2008) might reduce the degrees of population differentiation. In addition, the long-distance dispersal could also accelerate the gene flow among populations. During the last interglacial period, the Taiwan Strait formed, separating the populations of Taiwan and China, but the southward CCC and the northward SCSC and KBC might play roles as vectors to promote the dispersal of propagules among these populations. In addition, the submarine topography of the Taiwan Strait that affects the routes of currents also indirectly increases the cross-strait gene flow. For example, the Yunchang Ridge off central-western Taiwan could cause the northward SCSC and KBC to detour toward China, thereby causing the northward currents to turn toward northern Taiwan due to obstruction by the southward CCC (Huang and Yu 2003; Jan et al. 2002). The complicated current system of the Taiwan Strait may explain the mosaic genetic components of the Chinese populations revealed by Bayesian clustering analysis (Fig. 3).

Although we did not sample materials from the Ryukyu archipelagos, a previous study of microsatellite DNA and chloroplast DNA (cpDNA) sequences showed that the populations of the Ryukyus displayed relatively low genetic diversity and identical genotypes with the northern populations of Vietnam. Giang et al. (2006) studied the population genetics of *K. obovata* from the Iriomote population in the Ryukyu archipelago and two northern Vietnamese populations Dong Rui and Xuan Thuy, which are geographically close to DZG of Hainan Island. Their microsatellite data showed relatively lower genetic diversity in Iriomote than in the two Vietnamese populations and a high genetic differentiation between Iriomote and the Vietnamese populations (F_{ST}=0.207; 95%CI, 0.167–0.262). The lower genetic diversity (monomorphic) in Okinawa Island (Ryukyus) than in Dong Rui (four haplotypes) estimated based on the chloroplast *mat*K sequence (Kado et al. 2004) is consistent with the microsatellite estimation, although the small sample size is indicative rather than conclusive. These studies showed identical genotypes but a relatively lower genetic diversity in the

Ryukyus than in the southern populations, implying that the founders of Ryukyu populations were from the south. The route of colonization was probably driven by the northward currents from the tropic to the temperate, e.g., the SCSC and the Kuroshio Current. The latter carries warm water from the south to the north along the eastern Eurasian Continental Shelf and plays an important role in the demographic dynamics of East Asian organisms (Kokita and Nohara 2011; Liao et al. 2010; Yin et al. 2009).

Bottleneck effect and geographic-independent divergence inferred by outlier loci

The high frequency (13.57 %) of negative outlier loci in the populations of *K. obovata* suggests genetic hitchhiking due to background or balancing selection or sharing common ancestral polymorphisms among populations. Among these negative outliers, most loci (more than one third) were hitchhiked under balancing selection (Fig. S3, Online Resource). This means that the low F_{ST} is due to widespread ancestral polymorphisms, which break the migration-drift equilibrium, instead of purging variations by negative selection (cf. Manel et al. 2009). High degrees of relict genes of the past gene flow indicate strong linkage disequilibrium (Hamilton 2009) stemming from incomplete lineage sorting by the relative ages of the alleles in each locus (Chiang 2000), which is also revealed in the phylogenetic ambiguity of the dominant and widespread cytotypes of cpDNA and mitochondrial DNA in the genus *Kandelia* (Chiang et al. 2001).

In contrast to the negative outlier loci, the positive outliers, indicative of adaptation (divergent selection), are relatively rare (5.43 %). In addition to the adaptive effect, the high F_{ST} could also reflect a consequence of allele surfing (Klopfstein et al. 2006) during population expansion from a small population size, i.e., the bottleneck effect (Excoffier and Ray 2008; Hallatschek et al. 2007). Intermediate allele frequencies or high frequencies of rare alleles help in determining divergence among populations by local adaption or the rapid expansion of populations from a small size. The lack of intermediate frequencies of positive outlier alleles in SZ indicates that the alleles tend to be fixed or lost, revealing the phenomenon of rapid drift in a small population size (Fig. S3, Online Resource). In addition, SZ has relatively lower A among positive outliers than among neutral loci (P=0.0214, Student's t test), implying a high frequency of rare alleles and indicating rapid recruitment from the bottleneck. The demographic effect of the bottleneck in SZ is also revealed in its relatively low genetic diversity in both neutral and positive outlier loci (Table 2). Over-reclamation destroyed over 147 ha of mangrove area (about 48.8 % mangrove reserve area) along the coast of ShenZhen Bay during 1988–2000 (Guo et al. 2007), which might have caused a rapid decline in census population size and a severe bottleneck effect. In fact, the bottleneck effect was also revealed in the smallest effective population size in SZ than the other natural populations (Table 2).

In contrast to SZ, the other populations do not show significant differences in A between neutral and positive outlier loci and have an almost even distribution of allele frequencies of positive outliers (Fig. S3, Online Resource), suggesting that the divergence of positive outliers of these populations could be the effect of divergent selection or geographic isolation between populations. However, the divergent anonymous markers are probably not under selection themselves but are likely hitchhiked with undetected adaptive loci. In addition, Beaumont and Nichols's (1996) method for detecting the F_{ST} outliers, which are deemed to be adaptive loci could lead to large number of false positive (Meirmans 2012). On the other hand, the FD is distinct from two geographically close populations (ZJ and JW) in the first axis of the positive outlier PCoA (Fig. 4), and a highly significant positive correlation of heterozygosity was detected between the two geographically distant populations DZG and ZJ (Table S3, Online Resource), indicating that the divergence of positive outlier loci is not related to geographic distance (i.e., geography-independent divergence). Although we are unsure whether positive outliers were selected or not, the distinct genetic distribution of the positive outliers of JW and SZ, which have similar environmental properties (e.g., weather conditions, seawater properties, etc., Table S1, Online Resource), indicated that the selective pressures of the weather or seawater properties listed in the Table S1 might be unrelated to the population divergence (Fig. 4). Geography-independent divergence is also evidenced in the negative correlation between geographic and genetic distances in positive outliers (Fig. S4, Online Resource).

Biased sampling for nursery seedlings in reforestation

The conservation and restoration of mangroves is a common universal consensus among many countries, including China. The restoration and reforestation of mangroves were started decades ago in China. *K. obovata* was planted in YQ and LG from the southern coasts of Fujian Province of China. Since only the plantation in LG has source records (from FD in 1999 and 2008), we compared the genetic diversity of LG1 and LG2 with their source population FD. LG2 has significantly lower genetic diversity than FD and LG1 in neutral loci, but is not different with respect to positive outliers (Table 2), indicating that the genetic loss in restored populations is mainly affected by the drift effect rather than natural selection. Sampling bias during propagule collection during nursery processing and seedling production could alter the allele frequency of restored populations compared to the source population (El-Kassaby and Thomson 1996; Harlan 1975). Although we do not have detailed information about the practices of seed collection, the genetic loss in the restored

populations might indicate the biased sampling of seedlings from the source population. Expanded planting of the bias-sampled seedlings might have caused the bottleneck effect, resulting in lower genetic diversity, smaller effective population size (Table 2), and a distinct distribution of allele frequency in neutral loci (Fig. 4c). Although the census population size of threatened mangroves might increase by reforestation, the next step is to reconsider the sampling strategy of seed collection for genetic conservation. Such biased sampling of the seedlings for reforestation could even affect the allele frequency of natural populations by frequent pollen flow (Geng et al. 2008) and long-distance propagule dispersal by currents (cf. Liao et al. 2009), which would be counterproductive for conservation.

Conclusions

Colonization events of *K. obovata* can be traced back to the LGM of the Pleistocene period. The northward expansion of coastlines from the southern Taiwan Strait (the Penghu submarine canyon) through the ancient Minchiang waterway during the last interglacial period caused serial colonisations of *K. obovata* from its southern centre of origin. In addition, the early submergence of the continental shelf off northern Taiwan promoted colonization from the Ryukyus, which resulted in the grouping of northern populations in both Taiwan and China and differentiation from the South. The population structure of *K. obovata* is affected by both the geographic barrier (i.e., the Taiwan Strait) and different sources of colonizers (i.e., from the south and north). In addition, the topography of Taiwan Strait affected the population structure of natural populations. The genetic diversity of *K. obovata* was also influenced by the demographic fluctuation of local populations, such as the rapid expansion of SZ. Restoration of *K. obovata* with a biased sampling of nursery seedlings resulted in shifts in allele frequency and a structured population in LG. The anthropogenic founders of the restored population not only decreased the genetic diversity and effective population size itself but also altered the allele frequency of the natural population by gene flow. This study identified historical and topogeographical factors that affect the genetic diversity and population structure of *K. obovata* including demographic history, topography, and anthropogenic colonization.

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