Conserv Genet (2008) 9:191–199
DOI 10.1007/s10592-007-9324-0

RESEARCH ARTICLE

Demographic bottlenecks and low gene flow in remnant populations of the critically endangered *Berchemiella wilsonii* var. *pubipetiolata* (Rhamnaceae) inferred from microsatellite markers

Ming Kang · Jing Wang · Hongwen Huang

Received: 1 November 2006 / Accepted: 21 March 2007 / Published online: 18 April 2007

Abstract *Berchemiella wilsonii* var. *pubipetiolata* (Rhamnaceae) is an endangered plant with only four remnant populations in eastern China. Population genetic information is essential for understanding population history and formulating conservation strategies for this species. Thirteen microsatellite loci were used to investigate genetic variation and population structure of the four remnant populations. Moderate levels of expected heterozygosity (H_E = 0.466–0.543) and low allelic diversity (A = 3.1–3.6 and A_R = 2.2–2.4, respectively) were observed within populations. Bottleneck tests found three out of four populations to deviate from mutation-drift equilibrium under the two-phase model (TPM), suggesting a recent population decline, which is congruent with known demographic history. The evolutionary history of the species seems dominated by genetic drift rather than gene flow. Low historical gene flow was inferred from several different approaches and N_m ranged from 0.582 by the private allele method to 0.783 by the coalescent method. Contemporary gene flow was also found to be even lower for only one first generation migrant was detected with individual-based assignment analysis. Restricted pollen and seed dispersal as well as a recent decline in population size associated with habitat fragmentation may have contributed to low levels of historical and contemporary gene flow, and resulted in a high genetic differentiation. Under this scenario, *Berchemiella wilsonii* var. *pubipetiolata* populations are expected to display more pronounced population genetic structure in the future as a result of increased inbreeding and genetic drift.

Keywords Assignment test · *Berchemiella wilsonii* var. *pubipetiolata* · Equilibrium · Gene flow · Microsatellite

M. Kang · J. Wang
Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, Hubei 430074, China

H. Huang (✉)
South China Botanical Garden/South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China
e-mail: hongwen@wbgcas.cn

Introduction

Plant species with small and isolated populations are vulnerable to demographic, environmental and genetic stochasticity, and therefore face a higher risk of local extinction (Ellstrand and Elam 1993). Recent analyses of genetic variability and heterozygosity strongly demonstrate that maintaining genetic diversity is of importance as it is associated with population viability and the evolutionary potential of a species to respond to environmental change (Frankham et al. 2002; Reed et al. 2002; Reed and Frankham 2003). Demographic events such as decline of population size may lead to loss of genetic diversity because of reduction in gene flow between remnant populations, increased levels of inbreeding within remaining populations and higher incidence of genetic drift. While theoretical and experimental population genetic studies elucidate quantitative predictions about reduction in genetic diversity caused by population declines and bottlenecks (Nei et al. 1975; Frankham 1995; Cornuet and Luikart 1996), persistence of a small population may be further undermined by loss of allelic diversity, reduction in heterozygosity and the fixation of deleterious alleles. Consequently, management strategies for endangered species with small population size in isolated or fragmented

habitats must be designed to minimize genetic drift and inbreeding, so that processes that contribute to further loss of genetic variability can be halted. An understanding of demographic and genetic history and complex interaction between population demography and genetic connectivity is of great practical importance to conservation biologists in formulating conservation strategies for endangered species.

Berchemiella is a globally endangered genus of the Rhamnaceae family, including three species and one variety endemic to eastern Asia: *Berchemiella berchemiaefolia* (Makino) Nakai, *B. wilsonii* (Schneid.) Nakai, *B. wilsonii* (Schneid.) Nakai var. *pubipetiolata* Qian, and *B. yunanensis* Y. L. Chen et P. K. Chou (Qian 1988). Of those, *B. wilsonii*, *B. wilsonii* var. *pubipetiolata* and *B. yunanensis* are endemic to China, and *B. berchemiaefolia* is found only in Japan and Korea (Qian 1988). All taxa of the genus *Berchemiella* are designated as nationally endangered species in these countries (Kang et al. 1991; Fu and Jin 1992; Iwatsuki et al. 1999). *Berchemiella wilsonii* is an extremely endangered species listed on the China Plant Red Data Book (Fu and Jin 1992). The species has not been seen for almost a hundred years and has been treated as an extinct taxon in the wild since the first specimen was collected and described by E. H. Wilson in western Hubei Province, central China in 1907 (Fu and Jin 1992), until recently five isolated old trees were rediscovered in western Hubei.

Berchemiella wilsonii var. *pubipetiolata* Qian was first described in 1988 based on a distinct morphological difference from its progenitor *B. wilsonii* in having pubescent petioles (Qian 1988). It is a deciduous tree of up to 12 m and is only found in two disjunct regions: the northeastern Dabie Mountain in Anhui Province and the western Tianmu Mountain in Zhejiang Province (Qian 1988). *B. wilsonii* var. *pubipetiolata* was previously documented in at least seven sites (Qian 1988), however our recent field surveys have found only four remnant populations, with two in each region (Fig. 1). It is a weak competitor and occurs sparsely in the understory of forests in lowland drainage basins of streams and rivers at altitude between 500–1200 m above sea level. Pollination biology of this species has not been examined in great detail, but field reports and a preliminary allozyme study suggested that the species is a predominant outcrosser (Kang et al. 2005). Mature trees usually flower in late May or early June and seeds are dispersed by gravity, and occasionally by water.

The urgent need to elucidate appropriate management units for *B. wilsonii* var. *pubipetiolata* has led to several molecular marker-based genetic studies to investigate genetic consequences of fragmentation in its remnant populations (Kang et al. 2005; Kang et al. 2007). The previous allozyme study detected loss of low-frequency alleles in all remnant populations attributed to a recent severe decline in population size (Kang et al. 2005). A further combined analysis of AFLP and cpDNA markers detected strong genetic differentiation between two regional populations; hence they have been recognized as two evolutionary significant units (ESU) (Kang et al. 2007). Moreover, the significant inter-population differentiation revealed by both nuclear and cpDNA markers suggested that low levels of gene flow exist among the remnant populations (Kang et al. 2007). These previous studies however may have underestimated the genetic consequences of past demographic events as they used traditional standard approaches of assessing population structure (e.g. F_{ST}, a measure of allele frequency differences across populations) (Selkoe and Toonen 2006). Due to their hypervariability, codominance, reliable scoreability and applicability to rigorous statistical approaches, microsatellites have become one of the most popular genetic markers for ecology, evolution and conservation genetic studies (Sunnucks 2000; Zhang and Hewitt 2003; Schlötterer 2004; Selkoe and Toonen 2006). In the present study, we use newly developed polymorphic microsatellite markers to: (1) test deviation for mutation-drift and migration-drift equilibrium in remnant populations; (2) determine how demographic history is reflected in the current population genetic structure; (3) estimate levels of historical and recent gene flow between remnant populations of *B. wilsonii* var. *pubipetiolata*. Such information is of critical importance not only for delineating the genetic units for conservation and management, but also for a better understanding of factors that have shaped population structure of endangered plant species.

Materials and methods

Sampling and microsatellite genotyping

The sampling procedure of the populations and the study area has been described in previous study (for details see Kang et al. 2005): in short, a total of 98 individuals were collected from the four remnant populations (Fig 1). The DNA from nine individuals however failed to be amplified, thus a total of 89 individuals were analyzed in this study (Table 2). Thirteen microsatellite loci newly developed for this species (Bwp01, Bwp03, Bwp06, Bwp07, Bwp10, Bwp12, Bwp15, Bwp26, Bwp32, Bwp57, Bwp49, Bwp30, Bwp21) (Kang et al. 2006) were used for genotyping the 89 individuals and allele frequencies of each locus and for each population were estimated. The microsatellite procedure was performed as described by Kang et al. (2006).

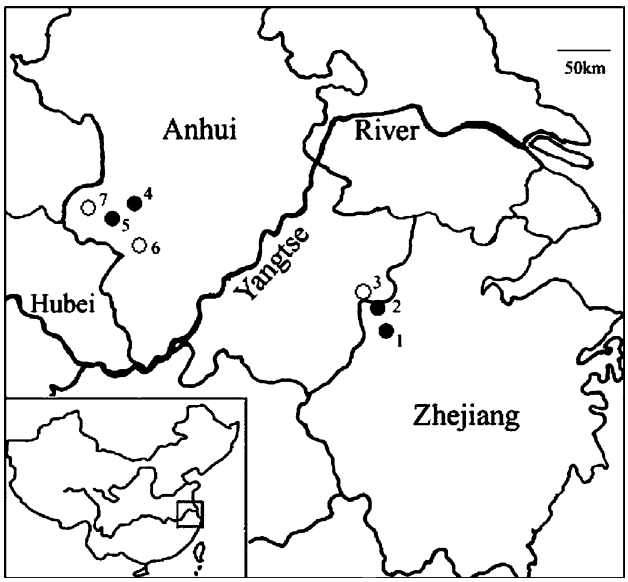

Fig. 1 Map of the historical and extant populations of *Berchemiella wilsonii* var. *pubipetiolata* and sampling locations (open circles, extinct sites; filled circles, remnant sites): 1, Tuankou; 2, Maxiao; 3, Jixi; 4, Wanfushan; 5, Majiahe; 6, Jinzai; 7, Qianshan

Statistical analysis

Genetic diversity within populations

A set of intra- and inter-population genetic statistics was calculated using following methods. GENETIX 4.05 (Belkhir et al. 1996-2004) was used to estimate: mean number of alleles per locus (A), expected (H_E) and observed (H_O) heterozygosity, and F_{IS} values for each population. Allelic richness (A_R) for each population was calculated using FSTAT 2.9.3.2 (Goudet 2001). This program was also used to test for deviation from Hardy-Weinberg equilibrium and linkage disequilibrium, with 1000 allelic permutations among individuals and a P value corrected for significance level ($\alpha = 0.05$) and multiple tests conducted with Bonferroni correction (Rice 1989).

Assessment of historical population bottlenecks

Genetic effects of the demographic decline of populations were examined by three approaches. The first approach is to detect recent population bottlenecks using the empirical approach described by Luikart and Cornuet (1998). The test relies on the assumption of selective neutrality and mutation-drift equilibrium; it is based on the principle that near the mutation-drift equilibrium the heterozygosity expected based on number of alleles and sample size (H_{eq}) equals the measured Hardy-Weinberg equilibrium heterozygosity (H_e) in non-bottlenecked populations. Instead, if a population decline is a recent event, the mutation-drift equilibrium would be temporarily disrupted and H_e will be significantly greater than H_{eq}, because the loss of alleles is faster than the reduction of heterozygosity (Luikart and Cornuet 1998; Piry et al. 1999). In the present study, we used the Wilcoxon sign-rank test of heterozygosity of excess under the stepwise mutation model (SMM), the infinite allele model (IAM), and the two phase model (TPM). Under the TPM, 70% of the mutations were assumed to occur under the SMM and 30% assumed to occur under the IAM. For each mutational model, 10 000 replicates were performed. The second approach was to test a mode shift away from an L-shaped distribution of allelic frequencies (Luikart et al. 1998). These two analyses were conducted using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999). The third approach that measures the mean ratio (M ratio) of number of alleles to the range in allele size (in SSR repeat units) (Garza and Williamson 2001) was implemented in the software $AGAR_{ST}$ (Harley 2002). In a population of constant size, the frequency distribution of most alleles is expected to be more or less continuous and the range in allele size similar to the number of alleles, so that, the mean ratio M would be close to one (Garza and Williamson 2001). During a population decline, the number of alleles decreases more rapidly than the range in allele size, leading to decrease of M (Garza and Williamson 2001). In comparison to the BOTTLENECK methods, M ratio reflects a population size decline over a longer time (Garza and Williamson 2001).

Genetic divergence between populations

The program FSTAT was used to calculate F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) across all populations. Pairwise population differentiation was evaluated by F_{ST} and R_{ST} using FSTAT and RSTCALC (Goodman 1997), respectively. Statistical significance of the estimates was determined with 1000 randomizations. The program POPULATIONS 1.2 (Langella 2000) was used to calculate Nei's (1983) genetic distance (D_A) among individuals. The principal coordinate analysis (PCO) was performed based on the resulting distance matrices using the DCENTER and EIGEN programs in the NTSYS pc 2.1 (Rohlf 2000).

Population structure was further examined using a full Bayesian-clustering approach, implemented in the program STRUCTURE (Pritchard et al. 2000). The first step in the analysis involves estimating the optimal number of clusters (K). To minimize the effect of the starting configuration during the Monte Carlo simulation, we simulated 5×10^5 burn-in period of the Markov chain before data for the parameter estimation were collected from a run length of 10^6 iterations. Five independent runs of $K = 1$–7 were performed to assure convergence of the chain and homogeneity among runs for each prior of K. Each run yielded a

log likelihood value, Ln *Pr* (*X*/*K*), the highest of which indicates the closest to the actual number of genetically distinct clusters. These analyses were based on an admixture ancestry model with correlated allele frequencies and assuming no prior information of population origin. After determining the number of clusters, this program was used to assign individuals into respective populations based upon proportional membership (q), and a threshold value of $q \geq 0.90$ was used.

Assessment of genetic drift and gene flow

The population history of *B. wilsonii* var. *pubipetiolata* was examined based on coalescent theory using the 2MOD program (Ciofi et al. 1999), which compares the relative likelihoods of a gene flow model *versus* a genetic drift model. The main assumption of both models is that the effects of microsatellite mutations are negligible. The gene flow model assumes mutation rate much smaller than the migration rate and the drift model assumes the reciprocal of the mutation rate is much larger than the migration rate. The same program was also used to estimate the Bayes factor (the ratio between the likelihoods of the gene flow model and the drift model) and the *F* statistic (the probability that two genes share a common ancestor within a population) for each population. The number of migrants per generation, *M*, in the gene flow model was calculated using the formula $M = (1–F)/(4F)$ (Ciofi et al. 1999). A Markov chain Monte Carlo (MCMC) simulation (10^5 iterations) was computed, with the first 10% of the output discarded in order to avoid bias to the starting conditions.

Historical gene flow (N_m) among populations was estimated indirectly from the formula $N_{m\ =}\ (1–F_{ST})/4F_{ST}$ (Wright 1969). In addition, we estimated gene flow using Slatkin's private allele method, which is based on the average frequency of unique alleles found in local populations (Slatkin 1985).

GENECLASS 2.0 (Piry et al. 2004) was used to detect putative first generation migrants, an indicative of recent gene flow. The likelihood ratio was estimated between the suspected immigrant individual and potential source populations using the Rannala and Mountain (1997) Bayesian method based on multilocus genotypes and the MCMC method with 1000 simulated individuals at a threshold of 0.05 (Paetkau et al. 2004).

Results

Microsatelliete variation and genetic diversity within populations

The 13 microsatellite loci used in this study generated a total of 71 alleles across the 89 individuals analyzed, ranging from three alleles at locus Bwp01 and Bwp49 to nine alleles at locus Bwp06 and Bwp10 (data not shown). The global mean expected (H_E) and observed heterozygosity (H_O) were 0.503 and 0.488, respectively. Significant deviation from Hardy-Weinberg equilibrium (HWE) was observed in six loci (Bwp03, Bwp10, Bwp26, Bwp57, Bwp30, and Bwp21). Similar levels of genetic variation as measured by A, A_R, H_E and H_O, were found in the all four populations (Table 1). Two populations (Maxiao and Wanfushan) showed significant deviation from HWE. No linkage disequilibrium was found for any locus pair in any population. A total of 19 private alleles were detected with 2, 7, 4 and 6 alleles specific to Tuankou, Maxiao, Majiahe and Wanfushan population, respectively (Table 2).

Deviation from mutation-drift equilibrium

Populations that have undergone recent population decline commonly exhibit an excess of heterozygosity, an evidence for deviation from mutation-drift equilibrium. Significant excess of heterozygosity was detected in all four populations under assumption of the IAM model, but only in three and one population under assumption of the TMP and SMM models, respectively (Table 3). The discrepancy between the three model tests was probably caused by different expected heterozygosities at mutation equilibrium

Table 1 Genetic diversity parameter estimates in four remnant populations of *Berchemiella wilsonii* var. *pubipetiolata*

Population	N	A	A_R	H_O	H_E	F_{IS}
Tuankou	23	3.1	2.3	0.486	0.499	0.027
Maxiao	13	3.6	2.4	0.509	0.543	0.105*
Majiahe	31	3.2	2.2	0.507	0.466	–0.070
Wanfushan	22	3.2	2.2	0.450	0.513	0.147**
Population mean	22.25	3.3	2.3	0.488	0.505	0.052
Overall samples	89	5.5	3.4	0.491	0.503	0.225

Sample size (N), mean number of alleles per locus (A), allele richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), fixation index (F_{IS}) and deviation from HWE tests, * Significant at $0.01 < P < 0.05$; ** Significant at $P < 0.01$

(Luikart and Cornuet 1998). Given that few microsatellite loci are expected to evolve strictly according to a one-step SMM, a combination of SMM and IAM, i.e. the TPM, is recommended (Di Rienzo et al. 1994; Spencer et al. 2000). In addition, three out of the remnant four populations displayed a shifted distribution of allele frequencies. Values of the *M* ratio varied between 0.487 and 0.610, which were less than 0.7, a criterion for showing bottlenecked populations (Garza and Williamson 2001). Combining the results from these different analytic approaches, it is reasonable to believe that recent demographic bottlenecks have occurred in remnant populations of *B. wilsonii* var. *pubipetiolata*, although it could not rule out the possibility that the results might be biased due to deviation from HWE in Maxiao and Wanfushan populations.

High genetic differentiation between populations

The global genetic differentiation across all populations estimated as F_{ST} and R_{ST} were 0.256 and 0.268, respectively. All pairwise values of F_{ST} (0.204–0.301) and R_{ST} (0.223–0.312) were highly significant ($P < 0.001$) (Table 4). The highest log-likelihood value was obtained for four clusters (Ln *Pr* (*X/K*) = –2084.4), indicating four genetically distinct populations of *B. wilsonii* var. *pubipetiolata*. At $K = = 4$, the results by STRUCTURE analysis revealed very high assignment accuracy for each cluster (0.962–1.000) (Table 5), suggesting strong differentiation existed among *B. wilsonii* var. *pubipetiolata* populations. The results are also congruent with the PCO plot of the 89 individuals (Fig 2), in which the first axis accounted for 31.1% of the total variation and separated the four populations into the two regions. The two populations Majiahe and Wanfushan in Dabie Mountain region were separated by the second axis, which accounted for 16.1% of the total variation.

Genetic drift and low gene flow

The relative likelihood of gene flow *vs.* drift models was estimated by the MCMC procedure as described in Ciofi et al. (1999). It revealed that a genetic drift model for populations of *B. wilsonii* var. *pubipetiolata* is more likely than the gene flow model [*P* (genetic drift) = = 0.999, Bayes factor = 1000], indicating the population structure of this species has been predominantly influenced by genetic drift rather than gene flow. High probabilities that two alleles were identical by descent within population (*F*) were observed, ranging from 0.179 for Maxiao to 0.277 for Tuankou (Table 3), with a mean value of $F = 0.242$. The mean indirect number of migrants per generation in each population (*M*) is 0.784.

The indirect estimates of historical gene flow, *N*m, inferred from F_{ST} and R_{ST}, are 0.727 and 0.683, respectively. The value of N_m calculated from the private allele method (Slatkin 1985) is 0.582. Obviously, all these estimates consistently demonstrate a low level of historical gene flow between the populations of *B. wilsonii* var. *pubipetiolata*.

Table 2 Private alleles found in populations of *Berchemiella wilsonii* var. *pubipetiolata*

Population	Locus	Allele	Frequency
Tuankou	Bwp06	230	0.239
	Bwp10	192	0.614
Maxiao	Bwp01	174	0.346
	Bwp03	158	0.167
	Bwp15	144	0.409
	Bwp26	180	0.115
	Bwp30	152	0.346
	Bwp30	160	0.192
	Bwp32	180	0.227
Maiiahe	Bwp01	178	0.038
	Bwp03	154	0.200
	Bwp12	160	0.017
	Bwp57	188	0.048
Wanfushan	Bwp06	196	0.045
	Bwp06	212	0.136
	Bwp21	178	0.136
	Bwp21	180	0.273
	Bwp26	200	0.409
	Bwp32	186	0.182

Table 3 Probabilities of allelic coancestry (F) by 2MOD and tests for mutation-drift equilibrium, probabilities from Wilcoxon sign-rank tests for heterozyosity excess and mode shift by using BOTTLENECK and M ratio values calculated by $AGAR_{ST}$

	Mutation-drift test					
	IAM	SMM	TPM	Mode shift	M ratio	F
Tuankou	0.0020**	0.0636	0.0043**	Shifted	0.569	0.277
Maxiao	0.0034**	0.6066	0.1878	Shifted	0.487	0.179
Majiahe	0.0009***	0.1018	0.0031**	Normal	0.610	0.245
Wanfushan	0.0001***	0.0012**	0.0001***	Shifted	0.477	0.266

** Significant at $P < 0.01$; *** Significant at $P < 0.001$

Table 4 Pairwise estimated values of F_{ST} (below diagonal) and R_{ST} (above diagonal) among remnant populations of *Berchemiella wilsonii* var. *pubipetiolata*

	Tuankou	Maxiao	Majiahe	Wanfushan
Tuankou	–	0.228***	0.266***	0.255***
Maxiao	0.204***	–	0.286***	0.312***
Majiahe	0.291***	0.262***	–	0.223***
Wanfushan	0.301***	0.276***	0.187***	–

*** Significant at $P < 0.001$

Table 5 The proportion of membership of each predefined population in each of the four clusters by using STUCTURE assignments

	Inferred cluster			
	1	2	3	4
Tuankou	0.999	0.001	–	–
Maxiao	0.037	0.962	–	–
Majiahe	–	–	0.999	0.001
Wanfushan	–	–	–	1.000

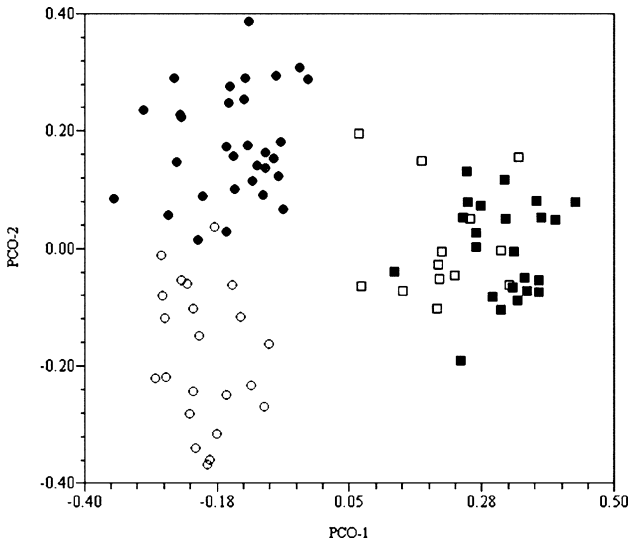

Fig. 2 Plot of the first two principal coordinates based on variation at 13 microsatellite loci showing *B. wilsonii* var. *pubipetiolata* individuals from Tuankou (■), Maxiao (□), Majiahe (●) and Wanfushan (○). The first two principal coordinates accounted for 31.1% and 16.1% of the total variation, respectively

Moreover, the assignment analysis detected only a single first generation migrant between the Tuankou and Maxiao populations, suggesting almost no contemporary gene flow occurring between remnant populations of the species.

Discussion

In comparison to the general trend of high microsatellite heterozygosity found in tree species (reviewed in Sanou et al. 2005), the results obtained in the present study indicate that the remnant populations of *B. wilsonii* var. *pubipetiolata* retain only moderate genetic diversity (expected heterozygosity H_E = 0.466–0.543, Table 1). This result is congruent with the previous AFLP analysis, which detected moderate genetic variation in this species when compared to many other endangered tree species (Kang et al. 2007). However, low allelic diversity, estimated by A and A_R was found in all of the four remnant populations (Table 1). For microsatellites, Spencer et al. (2000) suggested that allelic diversity is probably more

informative than expected heterozygosity for assessment of genetic erosion in populations, because allelic diversity is more sensitive than heterozygosity to the effect of demographic bottlenecks and reflects an immediate consequence underlined by genetic bottlenecks (Nei et al. 1975). Low allelic diversity and a lack of low frequency alleles ($P < 0.1$) were also found in allozyme loci of the same set of populations analyzed in this study (Kang et al. 2005). However, unlike in the microsatellite and AFLP markers, we found a surprisingly high-expected heterozygosity based on allozymes ($H_E = 0.348$, Kang et al. 2005) in the same set populations of *B. wilsonii* var. *pubipetiolata*. Natural selection for heterozygotes at allozymic loci may explain the discrepancy between nuclear DNA and allozyme markers. The DNA-based markers usually reveal higher genetic diversity than allozyme- based markers for the same species (Nybom and Bartish 2000), but lower DNA-based diversity was also detected in several plant species (e.g. *Medicago sativa*; (Jenczewski et al. 1999; and references there in). The observed low allelic diversity therefore might be a consequence of genetic erosion and a signature of recent genetic bottlenecks which have occurred in the remnant populations of this species.

Significant deviations for mutation-drift equilibrium under the IAM and TPM models for all and three out of four populations, respectively, suggest a recent decline in population size. The other two approaches (pattern of allele frequency distribution and *M* ratio) also provide additional evidence of recent bottlenecks occurred in populations of *B. wilsonii* var. *pubipetiolata*. Although low sample size in Maxiao and deviation from Hardy-Weinberg equilibrium observed in Maxiao and Wanfushan populations may bias the results obtained, the bottleneck tests in this study are likely to reflect the fluctuations in population size of this species. The anthropogenic disturbance on *B. wilsonii* var. *pubipetiolata* has attracted conservation attention since 1983 (Qian 1988). However, the threatened status of this species has not been ameliorated because of the rapid urbanization in eastern China. During the past several decades, *B. wilsonii* var. *pubipetiolata* populations have been subjected to severe deforestation, which resulted in habitat loss and local population extinction (Kang et al. 2005).

A high level of population differentiation was observed among all population pairs in the present microsatellite analysis (Table 4). The fully Bayesian assignments revealed that almost all individuals (mean 99%) were assigned to the population from which they were sampled (Table 5), suggesting restricted dispersal among the remnant populations. In addition, this result is in agreement with a large number of private alleles found in each population and the detection of only a single first-generation migrant between the populations Tuankou and Maxiao. A similar result was also obtained in the PCO analysis that revealed most individual trees clustered into distinct population-specific groups (Fig 2). High pairwise genetic differentiation between populations is probably a consequence of low gene flow among remnant populations and strong genetic drift occurred in these isolated populations, a hypothesis supported by the migration-drift equilibrium test. The values of historical gene flow estimated from private alleles (Slatkin 1985), population differentiation (F_{ST} and R_{ST}) and coalescence (F) methods are highly concordant ($N_m = 0.582–0.784$), and indicate low gene flow among *B. wilsonii* var. *pubipetiolata* populations. Taking into account the close geographic proximity within the regional populations (approximately 30 and 26 km for Dabie Mountain and Tianmu Mountain regions, respectively), the level of gene flow observed was also low between populations within regions (Table 4). Restricted gene flow in *B. wilsonii* var. *pubipetiolata* is seemingly not in accordance with the theoretical expectations for a long-lived, woody plant species with outcrossing breeding. However, evidence of restricted seed dispersal might explain the limited gene flow among populations of this species. Our field survey confirmed that seed dispersal of *B. wilsonii* var. *pubipetiolata* is very limited and is similar to its congener *B. berchemiaefolia*, where seed dispersal primarily occurs via gravity and occasionally by water (Lee et al. 2003). *B. wilsonii* var. *pubipetiolata* mostly occurs in lowland drainage basins of streams, and therefore seed migration is usually limited by geographic topography. Long-distance pollen dispersal may facilitate gene flow and mitigate the negative effects caused by restricted seed dispersal. For *B. wilsonii* var. *pubipetiolata*, however, pollen dispersal may only be effective within populations because of the low number of remnant populations and small size. Furthermore, habitat fragmentation caused by deforestation, road construction, ecotourism and urbanization may alter the behavior of pollinators (e.g. flight range of pollinators) and restrict pollen movement. Gene flow estimates of $N_m < 1$ in the present study imply that migration among *B. wilsonii* var. *pubipetiolata* populations is so rare that it will not counteract the effect of random genetic drift, as predicted by theoretical studies (Slatkin 1987). Consequently, increased levels of inbreeding within populations and genetic drift might occur and result in fixation of deleterious genes and reduced fitness of *B. wilsonii* var. *pubipetiolata*. The observed very low seed vigor and germination rate in this species (37.8% and 4%, respectively, Dang et al. 2005) seemingly indicate the deleterious genetic effects of inbreeding and drift undertaken in remnant populations.

In summary, the remnant populations of *B. wilsonii* var. *pubipetiolata* still retain a moderate level of genetic variation, as evidenced by both the present microsatellite

analysis and previous AFLP and chloroplast DNA analysis (Kang et al. 2007). We detected a loss of allelic diversity and deviations from mutation-drift and migration-drift equilibria in remnant populations of *B. wilsonii* var. *pubipetiolata*, which is likely to reflect anthropogenic disturbance and demographic history in this species. Our results indicate that populations of the species may have experienced a recent decline of population size. Under the present environmental circumstances and low gene flow between the remnant populations, populations of *B. wilsonii* var. *pubipetiolata* are expected to exhibit increased population genetic structure in the future and may become more susceptible to genetic drift.

The findings from this study have significant implications for the conservation management of *B. wilsonii* var. *pubipetiolata*. An effective management program should be formulated to incorporate following measures: (i) Based on genetic data that all sampled individuals characterized are unique molecular genotypes (AFLP and microsatellite), all extant individuals of *B. wilsonii* var. *pubipetiolata* should be effectively protected. Thus, capturing most of the genetic variation and monitoring remnant individuals and their derived progenies at each remnant population site become crucial for formulating in situ conservation programs. (ii) Establishing a well designated ex situ conservation site is urgent task. Previous efforts in preserving genomic integrity in ex situ conservation (Kang et al. 2005) should be rigorously evaluated to provide conservation guidelines for better ex situ practice. A more extensive collection of seeds from all individuals from each populations and enhancement of current ex situ conservation should be conducted so that random loss of genetic variants due to loss of individual ecotypes could be avoided. Further management measures should consider the establishment of new self-sustaining populations. (iii) Augmentation of each population by artificially propagated progenies of local individuals and re-establishment of the extinct populations using artificial progenies from the closest geographic proximity population are strongly recommended as a measure to increase effective population size and restore connectivity between remnant *B. wilsonii* var. *pubipetiolata* populations. (iv) Given the uniqueness of each remnant population, artificial crosses between individuals from populations within the same region are encouraged and microsatellite markers could be further used to select desirable parents. This could result in a highly heterozygous progeny array for purpose of selecting seedling with better adaptive fitness. These hybrid progeny arrays could be used to establish experimental populations whose adaptive fitness could be evaluated. Once the possible deleterious effects of such outbreeding have been screened out of the hybrid progeny, the artificial progeny array could be served as source for reinforcement and translocation in the natural range of *B. wilsonii* var. *pubipetiolata*.

Acknowledgements This work was partially supported by National Natural Sciences Foundation of China (30470185) and KIP Pilot Project of Chinese Academy of Sciences (KSCX2-SW-104). We likewise acknowledge support under WZ-060811 of Conservation Genetic Laboratory, WBG-CAS (Wuhan Botanical Garden, Chinese Academy of Sciences).

References

Belkhir K, Borsa P, Chikhi L et al (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France

Ciofi C, Beaumont MA, Swingland IR et al (1999) Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. Proc R Soc Lond B 266:2269–2274

Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Dang H, Zhang Y, Jiang M et al (2005) A preliminary study on dormancy and germination physiology of endangered species *Berchemiella wilsonii* (Schneid.) Nakai var. *pubipetiolata* H. Qian seeds. J Wuhan Bot Res 23:327–331

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA 91:3166–3170

Ellstrand NS, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Ann Rev Ecol Syst 24:217–242

Frankham R (1995) Inbreeding and extinction: a threshold effect. Conserv Biol 9:792–799

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. University Press, Cambridge

Fu LK, Jin JM (1992) China Plant Red Data Book- Rare and Endangered Plant. Science Press, Beijing, pp 538–539

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318

Goodman SJ (1997) RSTCALC: A collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol Ecol 6:881–885

Goudet J (2001) FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices Version 2.9.3.2, updated from Goudet 1995. Available from: http://www.unilch/izea/softwares/fstat.html

Harley EH (2002) AGAR$_{ST}$, version 2.8, a program for calculating allele frequencies, G_{ST} and R_{ST} from microsatellite data. Wildlife Genetics Unit, University of Cape Town, South Africa

Iwatsuki K, Boufford DE, Ohba H (1999) Flora of Japan, Vol. IIc. Kodansha Ltd., Tokyo, Japan

Jenczewski E, Prosperi JM, Ronfort J (1999) Differentiation between natural and cultivated populations of Medicago sativa (Leguminosae) from Spain: analysis with random amplified polymorphic DNA (RAPD) markers and comparison to allozymes. Mol Ecol 8:1317–1330

Kang M, Jiang M, Huang H (2005) Genetic diversity in fragmented populations of *Berchemiella wilsonii* var. *pubipetiolata* (Rhamnaceae). Ann Bot 95:1145–1151

Kang M, Xu F, Lowe A et al (2007) Protecting evolutionary significant units for the remnant populations of *Berchemiella wilsonii* var. *pubipetiolata* (Rhamnaceae). Conserv Genet 8:465–473

Kang M, Zhang J, Wang J et al (2006) Isolation and characterization of microsatellite loci in the endangered tree *Berchemiella wilsonii* var. *pubipetiolata* and cross-species amplification in closely related taxa. Conserv Genet 7:789–793

Kang SJ, Kim HE, Lee CS (1991) Ecological studies on the distribution, structure and maintenance mechanism of *Berchemia berchemiaefolia* forest. Korean J Ecol 14:25–38

Langella O (2000) POPULATIONS 1.2: population genetic software, individuals or population distances, phylogenetic trees. Available at: http://bioinformatics.org/project/?group_id = 84

Lee SW, Kim YM, Kim WW (2003) Lack of allozyme and ISSR variation in the rare endemic tree species, *Berchemia berchemiaefolia* (Rhamnceae) in Korea. Ann Forest Sci 60:357–360

Luikart G, Allendorf FW, Cornuet J-M et al (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Heredity 89:238–247

Luikart G, Cornuet J-M (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv Biol 12:228–237

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29:1–10

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. J Mol Evol 19:153–170

Nybom H, Bartish I (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Evol Syst 3:93–114

Paetkau D, Slade R, Burden M et al (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55–65

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Heredity 90:502–503

Piry S, Alapetite A, Cornuet J-M et al (2004) GENECLASS 2.0: A software for genetic assignment and first-generation migrant detection. J Heredity 95:536–539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Qian H (1988) A study on the genus *Berchemiella* Nakai (Rhamnceae) endemic to eastern Asia. Bull Bot Res 8:119–126

Rannala B, Mountain J (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197–9201

Reed DH, Briscoe DA, Frankham R (2002) Inbreeding and extinction: The effect of environmental stress and lineage. Conserv Genet 3:301–307

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

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Rohlf FJ (2000) *NTSYS-pc 2.1*: Numerical Taxonomy and Multivariate Analysis System, Version 2.1. Exeter Software, Setauket, New York

Sanou H, Lovett N, Bouvet M (2005) Comparison of quantitative and molecular variation in agroforestry populations of the shea tree (*Vitellaria paradoxa* C.F. Gaertn) in Mali. Mol Ecol 14:2601–2610

Schlötterer C (2004) The evolution of molecular markers – just a matter of fashion? Nat Rev Genet 5:63–69

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Slatkin M (1985) Rare alleles as indicators of gene flow. Evolution 39: 232–240

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Mol Ecol 9:1517–1528

Sunnucks P (2000) Efficient genetic markers for population biology. Trends Ecol Evol 15:99–203

Weir B, Cockerham C (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

Wright S (1969) Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago

Zhang DX, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol Ecol 12:563–584