Genetic diversity of *Meconopsis integrifolia* (Maxim.) Franch. In the East Himalaya–Hengduan Mountains inferred from...

**Article** in *Biochemical Systematics and Ecology* · December 2016

DOI: 10.1016/j.bse.2016.08.007

**CITATIONS**
0

**READS**
55

**6 authors**, including:

- **Jianwen Zhang**
  Chinese Academy of Sciences
  29 PUBLICATIONS  106 CITATIONS
  [SEE PROFILE]

- **Yong-Hong Zhang**
  Yunnan Normal University
  21 PUBLICATIONS  112 CITATIONS
  [SEE PROFILE]

Some of the authors of this publication are also working on these related projects:

- The evolution of plant diversity in *Crepidinae* (Asteraceae) [View project]

- Evolution and Biogeography of *Syncalathium* Complex [View project]

All content following this page was uploaded by Jianwen Zhang on 20 September 2016.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.
Genetic diversity of *Meconopsis integrifolia* (Maxim.) Franch. In the East Himalaya–Hengduan Mountains inferred from fluorescent amplified fragment length polymorphism analysis

Jian-Ling Guoa, 1, Xiao-Yun Zhanga, 1, Jian-Wen Zhangb, Zhi-Min Lia,c, Wen-Guang Suna, b, Yong-Hong Zhanga,*

a Life Science Department, Yunnan Normal University, Kunming 650500, China
b Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
c Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Kunming 650500, China

**Article info**

Article history:
Received 10 June 2016
Received in revised form 8 August 2016
Accepted 13 August 2016

**Keywords:**
*Meconopsis integrifolia*  
AFLP  
Genetic diversity  
BARRIER  
East Himalaya–Hengduan Mountains

**Abstract**

*Meconopsis integrifolia* (Maxim.) Franch. is a representative species of the genus found in the East Himalaya–Hengduan Mountain. As a species of medical and horticultural significance, *M. integrifolia* is threatened by over-exploitation and habitat fragmentation. In this study, the genetic diversity and structure of *M. integrifolia*, represented by 183 individuals from 10 populations, were studied using 6 pairs of fluorescent Amplified Fragment Length Polymorphisms primers. Our results showed a relatively high genetic variation at the species level (PPB = 82.0%, HE = 0.2356 and I = 0.3695). In contrast, population diversity was extremely low, as measured by Nei's genetic diversity index and Shannon's diversity index (HE = 0.0317, I = 0.0480). Analyses of molecular variance (AMOVA) showed that among and within populations, the genetic variation accounted for 88.9% and 11.1% of the total genetic variation, respectively. In addition, Nei's coefficient of differentiation (GST) was found to be high (0.8636), confirming the significant high level of genetic differentiation as well as low gene flow among populations of this species. The results of Neighbor-joining cluster, PCoA, and Bayesian assignment revealed similar genetic differentiation patterns, suggesting three main genetic groups that are concordant with their geographical distribution. Three main barriers—the Mekong River, the Yalong River, and a branch of the Yalong River—were detected using BARRIER software. The possible mechanisms and implications of these findings for conservation are discussed.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

*Meconopsis* Vig. is a well-known genus of flowers in the family Papaveraceae. The genus comprises 54 species that are mainly distributed in the Sino-Himalayan region, with one species, *M. cambrica* (L.) Vig., in west Europe (Zhang and Grey-
Wilson, 2008). The East Himalaya-Hengduan Mountains region is the center of diversity for the genus. *Meconopsis integrifolia* (Maxim.) Franch. is one of the most common members of the genus in the region. Its body is covered with long, barbellate hairs over entire margin leaf. There are 3–5 bright yellow flowers assembled in one inflorescence. These characteristics make *Meconopsis integrifolia* easily distinguishable from other species of the genus. This species is scattered in the alpine meadow or scree slope along mountain ridges, with an island-like distribution at altitudes of 4100–5100 m above sea level (Yang et al., 2012). For a long time, the whole plant was used as a traditional Tibetan medicine for treatments of various diseases, such as inflammation, pain, hepatitis and tuberculosis (Wu et al., 2011). In addition, this species is a beautiful wild flower and has been collected ornamentally from the field frequently. The species has been subjected to extensive collection and is used increasingly every year. Destruction of the species’ habitat resulting from overgrazing and overexploitation has led to the fragmentation of populations and a gradual decrease in their size and number (Chen et al., 2014).

Until now, studies of the genus *Meconopsis* focused on phytochemistry and pharmacology (Wu et al., 2011). Few studies were conducted on the phylogeny, breeding system, and germination (Chen et al., 2014; Liu et al., 2014; Wu et al., 2015). It has been reported that *M. integrifolia* has various chromosome numbers (n = 37, 41, 59 or 60, 76) (Ying et al., 2006) and a low seed germination rate (7.3%; Shi et al., 2008). A species’ level of genetic diversity determines its maintenance of populations for sustainable use. Understanding genetic variation within and among populations is essential to establish effective conservation practices. *M. integrifolia* is one of the most common species of the genus *Meconopsis* in the East Himalaya-Hengduan Mountains region. Therefore, it is crucial to understand the genetic variation within and among populations of this species and the factors that have shaped it to develop future improvements and conservation programs for this species and its congeners.

In the present study, we investigated the genetic diversity and population structure among ten populations representing the entire distribution range of *M. integrifolia* using the fluorescent AFLP fingerprinting technique. The main objectives of our study were (1) to reveal the amount of genetic diversity and how the genetic variation was distributed within and among the populations; (2) to detect possible factors affecting the genetic diversity and the genetic structure; and (3) to suggest methods for effective future conservation of this threatened alpine medicinal species.

2. Materials and methods

2.1. Plant materials

Sampling was conducted from 2010 to 2013 in ten *M. integrifolia* populations across the species’ distribution range (Fig. 1). In total, 183 individuals were collected, and sampled individuals were separated by at least 20 m. Table 1 shows the accession numbers, accession codes, localities and altitude for each of the populations. The collected leaf material was dried with silica gel in the field and maintained under -80 °C in the lab. Voucher specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

2.2. DNA extraction and AFLP analysis

Genomic DNA was extracted using the CTAB protocol established by Doyle and Doyle (1987). In addition, total DNA was cleaned using a DNA cleanup kit (Sunbiotech Ltd, Beijing, China). DNA concentrations were determined by comparing extracted samples with uncut lambda DNA on 1.5% agarose gels.

AFLP analysis was performed according to the method established by Vos et al. (1995) with a slight modification. Approximately 200 ng of genomic DNA was digested by an *EcoR* I and *Mse* I endonuclease mixture (New England Biolabs, Ipswich, MA, USA) for 3 h at 37 °C. The digestion was confirmed by electrophoresis on 1.5% agarose gels. Following heat inactivation of the restriction endonucleases, the restriction fragments were ligated to two adaptors, one for the *EcoR* I ends and one for the *Mse* I ends. The ligation mixture was incubated overnight at 20 °C. Pre-amplification of the diluted (10-fold) restriction-igation was conducted with primers (one selective nucleotide for each primer, A for the *EcoR* I primer and C for the *Mse* I primer) using the following cycling parameters: 30 cycles set at 94 °C for 30 s, 56 °C for 60 s and 72 °C for 60 s. The diluted (10-fold) pre-amplified products were used as the template for selective amplification.

Based on a preliminary assignment in which 24 pairs of selective primer combinations were tested eight times for polymorphisms and reproducibility, the whole sample set was proceeded using 6 primer combinations: E-AGC(FAM)/M-CTT, E-AGC(FAM)/M-CTA, E-AGC(FAM)/M-CTC, E-ACT(FAM)/M-CGT, E-AGC(FAM)/M-CAA, E-ACT(FAM)/M-CAC. The PCR program consisted of two segments. The first segment comprised 13 cycles with one cycle at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s. The annealing temperature was reduced by 0.7 °C per cycle during the first 12 cycles. The second segment comprised 23 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 60 s.

For each individual, 0.5 μl of selective PCR products was combined with 0.25 μl of the GeneScan ROX 500 (PE Applied Biosystems) internal size standard and 8.25 μl of formamide and run on a capillary sequencer ABI 3130XL (Applied Biosystems). Fragments in the ranges 100–500 bp were scored and exported as binary presence/absence matrices using GeneMarker Version 2.2 (Softgenetics, State College, USA) with manual checking. In total, 239 AFLP loci were identified and used in the analysis.
2.3. Data analysis

The genetic parameters were calculated using the POPGENE version 1.31 program (Yeh et al., 1997) assuming Hardy-Weinberg equilibrium. These parameters included the percentage of polymorphic loci (with 99% criterion); Shannon’s diversity index ($I$); Nei (1973) gene diversity ($H$); total genetic diversity ($H_T$); and the proportion of total diversity among populations ($G_{ST}$) (Nei, 1973, 1978). Then, the effective number of migrants per generation, which is an indirect estimate of gene flow between the two populations, was estimated by $N_m = 0.25 (1 - G_{ST})/G_{ST}$ (Wright, 1969).

Nei’s unbiased pairwise genetic distance data (Nei, 1978) was calculated by the program AFLP-SURV1.0 (Vekemans et al., 2002) and imported into PHYLIP (Felsenstein, 2005) for tree construction using the neighbor-joining (NJ) method. The AFLP-SURV program was also used to calculate 1000 bootstrap distance matrices, which were opened with the NEIGHBOR program in PHYLIP (Felsenstein, 2005) to obtain a tree file and elucidate relationships among populations. The CONSENSE program from the PHYLIP software was used to compute a majority-rule consensus tree.

The software STRUCTURE version 2.3.4 (Pritchard et al., 2000) was used to infer the number of biologically relevant clusters ($K$). Analysis of the number of clusters was performed using the non-admixture model with a burn-in and run lengths of 100,000 and 1,000,000 interactions, respectively. Analyses for each potential value of $K$, ranging from 1 to 10, were performed in 10 independent runs. The number of clusters was determined following the guidelines established by Pritchard et al. (2000) and Evanno et al. (2005).

AMOVA, analysis of molecular variance, was completed by GENALEX version 6.5 (Peakall and Smouse, 2012). It was used to estimate the components of variance attributable to differences among populations and among individuals within populations. Significance levels indicated by AMOVA were obtained following 1000 random permutations. To further assess genetic structure, two hierarchical principal coordinate analyses (PCoA) were also performed using the same software under population mode and individual mode.

To examine the geographic structure of genetic variation, we tested for correlations between genetic distance and geographic distance using a Mantel test (Mantel, 1967) based on the pairwise matrix of Nei, 1978 genetic distances and the pairwise matrix of geographic distances. A correlation coefficient ($r$) and one-tailed $P$ value were determined using 1000 random permutations. Furthermore, the genetic barriers associated with each geographical location and population were investigated using Monmonier’s maximum-difference algorithm (Monmonier, 1973) in BARRIER version 2.2 (Manni et al., 2004).
3. Results

A total of 239 unambiguous, repeatable bands were generated using the 6 selected primer combinations. Among these, 196 (82.0%) were polymorphic and included in the data matrix. The total number of bands scored per primer ranged from 35 (E-AGC/M-CTT) to 49 (E-AGC/M-CTA), with an average of 39.8 per primer combination (Table 2). At the species level, the percentage of polymorphic loci (NP), mean Nei’s gene diversity (HE) and Shannon’s index (I) of M. integrifolia were 82.0%, 0.2356 and 0.3695, respectively (Table 1). At the population level, the ten populations of M. integrifolia showed low levels of genetic diversity (Table 1). The percentage of polymorphic loci ranged from 3.4% to 19.7%, with an average of 10.2%. The number of polymorphic loci varied widely across the 10 populations, ranging from 8 in the population of Snow Mountain, Shangri-La county, Yunnan province (ZD), to 47 in the population of Gongga Mountain, Jiulong county, Sichuan province (JL), with an average of 24.4 (Table 1). The average Nei’s genetic diversity index (HE) and Shannon’s diversity index (I) were 0.0317 and 0.0480, respectively. The highest level of Nei’s gene diversity existed in the population from JL (HE = 0.0493), while the lowest was that from ZD (HE = 0.0143) (Table 1).

High genetic differentiation among populations of M. integrifolia was revealed by Nei’s method (GST = 0.8636). Genetic variability distribution among and within populations estimated by AMOVA revealed comparable results. AMOVA suggested that 88.9% of the total variation was from among populations, which was far more than that from within populations (11.1%). Genetic distances between populations of M. integrifolia varied from 0.0732 to 0.5180. The estimated gene flow among populations was extremely low (Nm = 0.0395), suggesting limited pollen and seed dispersal between populations of M. integrifolia. Populations ZD and DC were closest to each other in genetic distance, while populations DC and ZG were the most genetically distant. Our results revealed that the ZG population had the highest genetic differentiation from other populations (Table 1). The result of a Mantel test with 999 permutations revealed that the genetic divergence of populations (Nei’s genetic diversity) was significantly correlated with geographic distance in M. integrifolia (r = 0.4981, P < 0.01) (Fig. 2).

Our principal coordinate analysis (PCoA) based on Nei’s genetic distance revealed that the first three axes accounted for 30.5%, 22.3% and 17.5% of the total variance, respectively. Meanwhile, a similar result was obtained from the PCOa analysis based on Mean Population Genetic Distance Matrix for Binary Distance for each individual, with the three axes accounting for 26.0%, 20.3% and 13.5% of the total variance, respectively (data not shown). The ZG population, located in the west range of the Mekeong River, was clearly separated in plots of PCOa from other populations. Those populations (ZD, DC, YJJZW, BY and GZ) located between the Yalong River and the Jinsha River plotted together. The population YJGES plotted together with JL rather than with YJJZW, although it was located closer to YJJZW geographically. The populations SD and XJ showed separation from each other (Fig. 3). The NJ phylogram (Fig. 4) of populations showed that populations ZD, DC, YJJZW, BY and GZ clustered together. Those populations were located between the Jinsha River and Yalong River geographically. Population YJGES was clustered with population JL, although it was located closer to YJJZW geographically. The clades were distinct from each other with high bootstrap values (Fig. 4).

Table 2

<table>
<thead>
<tr>
<th>AFLP primers</th>
<th>Total number of bands</th>
<th>Polymorphic bands</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-AGC/M-CTT</td>
<td>35</td>
<td>29</td>
<td>82.9</td>
</tr>
<tr>
<td>E-AGC/M-CTA</td>
<td>49</td>
<td>37</td>
<td>75.5</td>
</tr>
<tr>
<td>E-AGC/M-CTC</td>
<td>42</td>
<td>39</td>
<td>92.9</td>
</tr>
<tr>
<td>E-ACT/M-CTT</td>
<td>37</td>
<td>24</td>
<td>64.9</td>
</tr>
<tr>
<td>E-AGC/M-CAA</td>
<td>37</td>
<td>34</td>
<td>91.9</td>
</tr>
<tr>
<td>E-ACT/M-CAC</td>
<td>39</td>
<td>33</td>
<td>84.6</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>196</td>
<td>82.0</td>
</tr>
</tbody>
</table>

Structure software, which analyzes genetic structure through a Bayesian approach, showed that the number of genetic groups (K value) best fitting our data, inferred using the method established by Evanno et al. (2005), was K = 3 (Fig. 5), with
most individuals from the populations located between the Yalong River and the Jinsha River being included in Group 1 and most individuals from the most western population ZG being in Group 2. Those individuals from eastern populations (JL, YJGES, XJ, SD) were separated into Group 3. Geographic barriers among populations and genetic boundaries between populations supported the distinctions between the three groups, as shown in Figs. 1 and 6. The first boundary isolated only one population (ZG) from the other populations, with 100% bootstrap support. The second boundary was found between populations located at the western and eastern side of Yalong River. The third boundary separated the SD population, which was located at the watershed between the Yalong and Dadu rivers, from JL, YJGES, and XJ populations with >80.0% bootstrap values. Meanwhile, one faint boundary was detected between populations JL and YJGES and population XJ. The Dadu River acted as the geographical barrier and separated the three populations into two sub-groups with a relatively low bootstrap value (29.5%).

4. Discussion

4.1. Genetic variation of M. integrifolia and its possible causes

Genetic variation at the intraspecific level is critical for adaptation to environmental changes and the long-term survival of a species (Schaal et al., 1991). Previously, it was reported that M. integrifolia was self-compatible, with approximately 65.0% set seed by autonomous self-pollination. Both flies and thrips were observed as pollinators for this plant, with flies moving and transferring pollen between plants and thrips moving within flowers, resulting in “facilitated selfing” (Wu et al., 2015). Seeds are released when the capsules are dry and dehiscent. Large amounts of seeds are often produced from a single plant and are numerous from each capsule. Seeds are small sized (ca. 1.0–1.5 mm long and 1.0 mm in diameter) and relatively light (mass of 1000 grains ca. 0.7481) (Chen et al., 2014). The seed coat is glabrous without any apparent epizoic adaptations. Seeds are probably dispersed over short distances via gravity. All of the traits listed above are associated with low or moderate genetic diversity (Hamrick et al., 1979; Hamrick and Godt, 1996). However, the level of genetic variability of M. integrifolia obtained in this study is relatively higher than the average values for plants with mixed-mating system and seed dispersal with gravity ($H = 0.174$; Hamrick and Godt, 1996). It has been suggested that the mean levels of genetic variation are lowest in narrow endemics, that they increase in regional endemics, but that they then decrease in widespread species (Hamrick et al., 1979). At the same time, long-lived perennial species usually have higher genetic variation than annual, biannual and short-lived perennial species (Hamrick et al., 1979). M. integrifolia is a perennial herb with patchy distribution in the East Himalaya-Hengduan Mountains region. Both factors might contribute to the increasing genetic diversity in M. integrifolia.

Though higher than the average genetic diversity reported previously (Hamrick and Godt, 1996), the results of this study revealed lower genetic diversity in M. integrifolia than in several reported plant species with similar distribution using AFLP method, such as Aconitum kongboense Lauener ($H = 0.525$, $I = 0.285$; Meng et al., 2014), Buddleja crispa Bentham ($H = 0.3135$, $I = 0.4851$; Zhang et al., 2015), and Fritillaria cirrhosa Maxim ex Batal ($H = 0.3275$, $I = 0.5414$; Zhang et al., 2010). It is even lower than the reported genetic diversity of congeneric M. quintuplinervia Regel ($H = 0.2954$, $I = 0.4371$; Yang et al., 2010). In addition, we detected unexpected, extremely lower levels of genetic variation within populations of M. quintuplinervia ($H = 0.0317$, $I = 0.0480$) than within population of M. quintuplinervia ($H = 0.2408$, $I = 0.3347$).

M. quintuplinervia is distributed on Qinghai-Xizang plateau (S Gansu, W Sichuan, SE Qinghai and E Xizang) in the northwest of China at altitudes from 1800 to 4600 m with a very broad ecological amplitude, growing commonly in low scrub and mountain meadows and at the edge of forests (Yang et al., 2010). The broad ecological amplitude might contribute to creating and maintaining the observed high level of genetic variability in M. quintuplinervia (Yang et al., 2010). Meanwhile, populations of M. integrifolia are scattered in the marginal alpine meadow or scree slope along mountain ridges at altitudes of
4100–5000 m, with an island-like distribution. The small population size can reduce genetic variation through genetic drift and reduce the inherent material for evolutionary changes (Boyce, 1992), which makes it more difficult to recover from occasional cataclysms and, in turn, further enhances the effect of genetic drift. Thus, genetic drift, together with inbreeding caused by the small population size, may have contributed to the observed low genetic diversity in *M. integrifolia*.

Historical factors, especially the Quaternary climatic oscillations in the Himalaya-Hengduan Mountains region, may also play a role in shaping its population genetic variation. Climatic oscillations caused repeated retreats and advances of many species’ ranges, resulting in shifts in the dominant vegetation during glacial and interglacial cycles (Hewitt, 2000). Specifically, as a cold-adapted species, the historical distributions of *M. integrifolia* may retreat to lower altitudes during ice-ages and colonize to higher altitudes as the temperature increased during the interglacial period. Thus, the frequent extinctions and recolonizations of local populations may have contributed to the low level of genetic diversity (Zhu et al., 2009).

### 4.2. Genetic structure and gene flow barrier

Genetic differentiation among populations is the integrated reflection of a long evolutionary history and includes factors such as shifts in distribution, habitat fragmentation and population isolation, as well as gene mutation, genetic drift, breeding system and gene flow (Hamrick and Godt, 1996; Nybom and Bartish, 2000). This study found high genetic differentiation among populations of *M. integrifolia* ($G_{ST} = 0.8636$) estimated by Nei’s method. AMOVAs of the AFLP markers revealed similar patterns of genetic structure, with 88.9% of the total variation among populations of *M. integrifolia*.

![Fig. 3. Principal coordinates analysis of the AFLP data from 10 populations of *M. integrifolia*. The first and second axes explained 30.5% and 22.3% of the total genetic variance, respectively.](image)

![Fig. 4. Unrooted neighbor-joining phylogram based on Nei, 1978 genetic distance data with bootstrap values.](image)
It had been suggested that the pattern of the genetic structure of a certain species is dependent on the ratio of self or inbreeding (Loveless and Hamrick, 1984), with inbreeding species usually exhibiting higher degrees of genetic differentiation than species with mixed breeding or with outcrossing (Nybom and Bartish, 2000). Wu et al. (2015) observed mixed-mating systems with an approximately 65.0% set seed by autonomous self-pollination. High levels of inbreeding might contribute to the high genetic differentiation.

Loveless and Hamrick (1984) suggested that limited pollen movement and local foraging, especially by small insects, can increase differentiation. Consistently, Avise (2004) reported that plants pollinated by animals with a weak flight ability would have significant population genetic differentiation. *M. integrifolia* was pollinated by flies and thrips, especially by thrips that always move within flowers (Wu et al., 2015). This finding might explain the high level of genetic differentiation among populations because the activity of the pollinator was limited, and thus, pollen exchange among populations was reduced. In addition to pollen exchange, gene flow induced by seeds is another important factor that influences genetic differentiation among populations. Plants whose seeds were dispersed by gravity would have higher genetic differentiation (Loveless and Hamrick, 1984). Though the seed dispersal of *M. integrifolia* is not clearly understood, the lack of ability to disperse over long distances with the aid of wind or animals will potentially limit gene flow and promote genetic differentiation among populations.

Additionally, most populations of this plant are distributed in the East Himalaya-Hengduan Mountains region. Therefore, the series of parallel mountain ranges dissected by deep river valleys that run from north to south could act as physical barriers and obstruct effective gene flow among populations of *M. integrifolia*, thereby enhancing population differentiation.

According to previous phylogeographic studies, the mountains ridges in the East Himalaya-Hengduan Mountains region constituted genetic barriers for plants and animals (Chen et al., 2010; Li et al., 2011; Zhang et al., 2015). For example, genetic diversity and estimates of population subdivision were markedly different for populations growing on either side of the “Mekong-Salween Divide” (Li et al., 2011). Other studies have shown the effectiveness of rivers as barriers to gene flow in some animals (e.g., *Scutiger boulengeri* Bedriaga (Li et al., 2009), *Ichthyophis bannanicus* Yang (Wang et al., 2015)). However, for plant species, the barrier effect of rivers is uncertain.

In this study, three main boundaries were detected using the BARRIER software (Manni et al., 2004) with Monmonier’s maximum-difference algorithm (Monmonier, 1973). The first boundary was located between the western population ZG and eastern populations ZD, DC, YJJZW, GZ, and BY. The Mekong and Jinsha Rivers may serve as a geographical barrier. Structure analysis based on the Bayesian clustering algorithm assigned individuals from the ZG population to an independent gene pool. PCoA analysis and NJ cluster analyses also revealed similar genetic differentiation patterns. The Yalong River served as a second barrier. Two neighboring YJGES and YJJZW populations had been placed into two different gene pools that were separated by the Yalong River. Though the ZD population is distant from the GZ population geographically, they shared the same gene pool, clustered in the same clade, and plotted closely in Structure analysis, NJ clustering analysis, and PCoA analysis. Hence, we proposed that the barrier effect of rivers might play an important role in the high genetic differentiation of *M. integrifolia*.

### 4.3. Implication of the genetic information for conservation

*M. integrifolia* is an important Tibetan medicinal herb with a high content of alkaloids; it is used to treat hepatitis, pneumonia, and edema (Zhou et al., 2013). All materials for medical usage are gathered from wild collection. Meanwhile, the
collection for ornamental use is another threat to its population maintenance. In recent years, over-collection has led to a severe decrease in population. In situ conservation is most effective as whole gene pools are protected in their natural habitat (Poudel et al., 2014). Therefore, collection in the wild should be strictly prohibited, and medicinal supplies of this species should also be restricted to commercial utilization. In this study, our analysis revealed three gene pools isolated by geographical barriers (Fig 6) with little genetic exchange or no migrants. Therefore, they should be considered as distinct management units (MUs; Moritz, 1994) that require separate monitoring and management. Because of the high population differentiation in the genetic structure of M. integrifolia ($\theta = 0.8636$), maintenance of a limited number of populations in such cases is not sufficient to preserve the major diversity at the species level. To conserve the genetic diversity of M. integrifolia, it is necessary to preserve as many populations as possible in the wild. Ex-situ conservation is another efficient strategy for genetic diversity protection that can be carried out with living plants in botanical gardens and tissue culture units through farming and as seeds in seed banks. In practice, high potential risks of genetic erosion and bottlenecks might occur due to the founders used in restoration from limited sources (Hedrick, 2000). In this regard, as much of the natural ranges as possible should be covered at the outset. At the very least, high priority should be placed on maintaining representatives from three of the identified groups.

Acknowledgments

We thank Jian Hong-Ying and Ma Lu-Lin of Flower Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China for their help in collecting plant material of M. integrifolia. This work was supported by the National Natural Science Foundation of China (grant no. 31160045, 31460050, 31360049, 31110103911), Main Direction Program of Knowledge Innovation of Chinese Academy of Sciences (grant no. KSCX2-EW-Z-1) and West Light Foundation of the Chinese Academy of Sciences.

References


