Article

Effects of Landscape Fragmentation on Genetic Diversity of Male-Biased Dioecious Plant *Pistacia chinensis* Bunge Populations

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Abstract: *Pistacia chinensis* Bunge (Anacardiaceae) is a dioecious woody plant of significant economic values that is used in traditional Chinese Medicine as well as for wood production. More importantly, it is one of the ideal tree species for bio-diesel production because of the high oil content in its seeds. In this study, we aim to reveal the effects of landscape fragmentation on the genetic diversity (GD) of the dioecious plant *Pistacia chinensis* populations. A total of nine microsatellites were used to genotype 180 *P. chinensis* individuals from six populations to estimate the differences in GD between different populations. The study revealed that genetic diversity of the *P. chinensis* population as a whole is relatively high in the Thousand-Island Lake (TIL) region, but its fragmented landscape still led to the loss of rare alleles, especially in a fragmented small population, a post-fragmented population, and a male population. The partitioning of a large continuous population into small isolated remnant patches led to the direct loss of genetic diversity and, subsequently, because of the mediated gene flow of seeds and pollen, genetic drift, and the spatial distribution of existing plants, the GD gradually decreased. The restricted gene flow and the increase in self-pollination and inbreeding impaired the population's long-term development. Therefore, the wild *P. chinensis* populations in the TIL region needs effective protective measures, including foreign artificial pollination and seedling transplantations.

Keywords: dioecious plant; *Pistacia chinensis*; sex ratio; landscape fragmentation; genetic diversity

1. Introduction

Landscape fragmentation because of human activities and geological processes has decreased the habitat size and increased the spatial isolation of habitats, leading to the fragmentation of populations into smaller ones [1–3]. The consequences of population fragmentation are multifold, but generally lead to a loss of genetic diversity and an increase of population differentiation, which are associated with a reduction in population size and an increase in inbreeding [4–7]. These negative genetic consequences can contribute to the loss of fitness and viability in plant populations.

Dioecious plants are plants that have male and female reproductive systems in separate plants. Females often invest more energy and nutrients in flowers and fruits, and less in growth and maintenance, whereas males invest only in flowers [8,9]. These different investment priorities between the sexes may result in contrasting survival rates and distinct growth patterns under long-term fragmentation conditions. Many studies have shown that landscape fragmentation may lead to a biased sex ratio in dioecious plant populations [10,11]. Biased sex ratios may further influence the genetic diversity, but the consequences may be different for different species [12–14]. Vandepitte et al. [15] observed that genotypic diversity decreased with more male-biased sex ratios in the dioecious forest

perennial *Mercurialis perennis* L. Hilfiker et al. [12] studied the small populations experiencing genetic drift and revealed an increased sex ratio bias toward females in the dioecious conifer *Taxus baccata* L. Thus, dioecious plants may differ greatly in their responses to landscape fragmentation. Further research on dioecious plant populations will help to increase our understanding of variations in the response of dioecious plant populations to fragmentation.

The objective of this study is to determine the impacts of fragmentation on the genetic diversity (GD) of *P. chinensis*, which is a dioecious plant with first flowering time being about 6 years. For this research, we chose an artificial reservoir formed 60 years ago, the Thousand-Island Lake (TIL), which provides a good study site for both spatial and time scales. We examined the before and after fragmentation cohorts from the same populations, similar to that studied by Bashalkhanov et al. [16] to investigate the genetic effects of air pollution on *Picea rubens* Sarg. Using nuclear microsatellite markers to genotype each individual and comparing the GD patterns of the populations, we aimed to answer the following questions: (1) Does landscape fragmentation affect GD of *P. chinensis* populations? (2) Do sex-biased populations differ in GD?

2. Materials and Methods

2.1. Study Area

The TIL (29°22' N to 29°50' N and 118°34' E to 119°15' E) is a large artificial reservoir located in Southeastern China (Chun'an County, Zhejiang Province), which was formed after the construction of the Xin'an River Hydroelectric Power Station in 1958. The lake contains 1078 islands with areas larger than 2500 m² and covers 60 km from east to west, and 50 km from north to south. It has a maximum depth of 108 m and an average of 34 m. Its surface area is 583 km² and the island area is 409 km². The terrain around the lake is complex, consisting primarily of low mountains and hills. The vast majority have altitudes between 120–600 m, while the highest elevation is 967 m. The zonal soil has medium thickness and fertility. Because of its location at the northern edge of the subtropical monsoon zone, the area has distinct seasons, with abundant sunshine and rainfall. The TIL possesses 1824 species of vascular plants, including 20 species that are under national protection, with a forest coverage rate of 95%. The TIL was declared a National Forest Park in 1986 [17,18].

In this study, we selected a 100 km² area in the north-central quadrant of the TIL area as the research site. The landscape pattern has changed substantially after the formation of the reservoir over 60 years ago. The original land, which was mainly mountainous, was covered by water, and the remaining land formed islands and peninsulas. Because of the largeness of the TIL region, the simultaneous fragmentation of habitats, and the high degree of isolation by water, this area is an ideal field site for studying the effects of landscape fragmentation on plant populations [19].

2.2. Study Species

Pistacia chinensis B. is a dioecious, wind-pollinated deciduous species [20]. *P. chinensis* is distributed widely in China and can be found in 26 provinces, municipalities, and autonomous regions [21]. *P. chinensis* is not only an excellent timber, ornamental, medicinal, and oil tree, but is also a highly economical species that drives a variety of industries (Figure 1a,b) [22,23]. The flowering months of *P. chinensis* are March and April, and seeds mature in September and October (Figure 1c–e) [20]. Wild *P. chinensis* first produces blossom generally between the ages of 6 and 10 years, with male plant development occurring earlier than the females [24]. Wild *P. chinensis* populations were discovered to exist on many islands during a plant survey in the TIL region (Figure 1a) [17]. The wild plants prefer shade and have certain requirements for soil moisture and soil thickness. *Pistacia chinensis* is reproduced by seeds, not by cloning, every tree is genetically distinct.

Figure 1. *Pistacia chinensis* B. (a) *P. chinensis* population, (b) *P. chinensis* tree, (c) male flowers, (d) female flowers, (e) seeds, and (f) the collection of fresh intact leaves.

2.3. Field Sampling and Data Collection

Based on the size of the island area and the distribution of the *P. chinensis* populations [9], three types of habitats were established: (1) mainland habitats A1 and A2; (2) large island habitats B1 and B2; and (3) small island habitats C1 and C2, to study the effects of landscape fragmentation on GD in the dioecious plant populations of *P. chinensis* (Figures 2 and 3). The location of each of *P. chinensis* plant we surveyed was documented using a portable global positioning system (GPS). Each *P. chinensis* plant was marked with wide red tape, and the site, number, and gender carefully recorded. We selected 20–40 individuals in each population that displayed normal growth and had no obvious defects. From each plant, we collected 6–8 fresh intact leaves and covered them with absorbent paper. We then submerged the leaves in blue silica gel, one leaf per zip lock bag, to ensure rapid dehydration (Figure 1f). The silica gel was changed regularly to keep the blades fresh. Because *P. chinensis* easily bifurcates, we measured the plant basal diameter. If there were no split ends, then we also measured the diameter at breast height (DBH). We used the growth standards of *P. chinensis* and the DBH growth curve to infer the age of each individual and classified each tree as seeding before or after fragmentation [24]. We acquired a total of 180 samples, including 60 pre- or post-fragmentation individuals, 43 females, and 66 males in three large populations (Table 1).

Figure 2. Map indicating the locations of the six *Pistacia chinensis* populations. Large island habitats B1 and B2; and small island habitats C1 and C2.

Figure 3. Isolated tree stands on islands in the Thousand-Island Lake of China.

Table 1. Population size, sex ratio (male/total), and sample size of each *Pistacia chinensis* population, as well as the number of pre- and post-fragmentation individuals, number of males, females, and seeding individuals in the northern part of Thousand-Island Lake, China.

Population	GPS	Population Size	M/T	Sampling Area (ha)	Sample Size	Pre	Post	Male	Female	Seedling
Pop A1	29°38′02.022″ N 118°57′20.046″ E	500	0.525	2.574	40	23	17	20	18	2
Pop A2	29°35′32.850″ N 118°54′13.266″ E	85	0.538	0.684	20					
Pop B1	29°35′07.554″ N 118°55′30.702″ E	162	0.626	1.207	40	21	19	23	14	3
Pop B2	29°34′13.387″ N 118°55′19.549″ E	61	0.6	0.685	20					
Pop C1	29°35′01.591″ N 118°55′07.459″ E	163	0.675	0.802	40	16	24	23	11	6
Pop C2	29°34′50.882″ N 118°55′21.380″ E	30	0.7	0.278	20					

Abbreviations: GPS, global positioning system.

The topography of the sampling area is mountain and hilly with a slope of 15–45 degrees. The soil is mountain red soil and sub-class of yellow-red soil. Their parent materials are acid magmatic rocks and sandstone weathering bodies. Soil pH is 4.5–5.0 and soil moisture is 8–12%. Soil N contents of each sampling population were A1, A2: 4.21 mg/g, B1: 4.99 mg/g, B2: 4.25 mg/g, C1: 4.93 mg/g, and C2: 4.41 mg/g, respectively. Generally, compared with small populations, habitats of large populations are better, they have deeper soils which are fertile and have weaker acidity and less steep slopes.

2.4. DNA Extraction and Microsatellite Genotyping

Genomic DNA was extracted using the Plant Genomic DNA Kit (Tiangen, Beijing, China), and its concentration and purity was measured using a micro-ultraviolet spectrophotometer. A total of nine polymorphic microsatellites, PTMS-3, PTMS-7, PTMS-9, PTMS-10, PTMS-33, PTMS-40, PTMS-41, PTMS-42, and PTMS-45, suitable for *P. chinensis* were selected from primers that were designed for *Pistacia vera* L. [25]. The forward primers for each microsatellite were modified to contain different fluorescent color tags, such as ROX, HEX, and FAM, on the 8909 automatic DNA synthesizer

(ABI Company, Carlsbad, CA, USA). DNA amplifications in a 10 μ L reaction system included 1.8 μ L DNA template, 0.1 μ L forward primer, 0.1 μ L reverse primer, 4 μ L 2 $\times$ master mix, and 4 μ L ddH₂O. The polymerase chain reaction (PCR) program used was 3 min denaturing at 94 °C, followed by 45 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C, and 2 min extension at 72 °C, and ending with a 10 min final elongation stage at 72 °C. The PCR products were detected and analyzed using an ABI 3730 xl sequencer with the LIZ (internal lane standards) 500 standard. Fragment lengths were obtained using the software GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical Analysis

Genepop 4.0.7 [26] was used to test for deviations from linkage disequilibrium (LD), and Micro-Check 2.2.3 [27] was used to test for null alleles and departures from the Hardy-Weinberg equilibrium (HWE). To characterize the GD within each population, the number of alleles (N_a), the effective number of alleles (N_e), the expected heterozygosity (H_e), and the observed heterozygosity (H_o) were analyzed with GenAlEx 6.5 [28]. In addition, estimates of allelic richness (AR, standardized for 20 individuals) and private rare allelic richness (PAR) were calculated using HP_Rare 1.1 [29]. Genetic differentiation was evaluated by F_{ST} , gene flow (N_M), and the inbreeding coefficient (F_{IS}) using FSTAT 2.9.3.2 [30].

The statistical terms were defined as the following:

N_a = number of alleles

N_e = effective number of alleles based on the Simpson's index

H_e = expected heterozygosity

H_o = observed heterozygosity

Allelic richness (AR): Allelic richness (number of alleles) is a measure of genetic diversity indicative variation in allele numbers.

Private rare allelic richness (PAR) was calculated from the rare allele data using the rarefaction methods.

Gene flow = N_M

Inbreeding coefficient = F_{IS}

F_{ST} : Wright's F-statistics, and especially F_{ST} , provide important insights into the evolutionary processes that influence the structure of genetic variation within and among populations, and they are among the most widely used descriptive statistics in population and evolutionary genetics.

3. Results

3.1. Microsatellite Loci

The results of linkage disequilibrium (LD) tests showed that all nine microsatellite loci did not have linkage relationships in the six populations of *P. chinensis*, indicating that the nine loci are inherited independently. Therefore, all nine loci were retained for a further analysis of variation in *P. chinensis* populations. Meanwhile, the null alleles test revealed no evidence of null alleles. After Bonferroni correction, three loci departed slightly from HWE (Table 2), possibly because of a deficit of heterozygotes (locus Ptms41). Using the nine microsatellite loci, we detected 140 alleles in 180 individuals, and the N_a per locus ranged from 10 (locus Ptms 3) to 25 (locus Ptms 41), with an average of 15.6 alleles. The H_o and H_e of the loci, estimated over all populations, ranged from 0.483 to 0.883 and from 0.438 to 0.780, respectively (Table 2).

Table 2. Genetic characteristics of nine microsatellite loci in six *Pistacia chinensis* populations containing a total of 180 individuals.

Locus	Na	Ho	He	HWE
Ptms3	10	0.533	0.720	ns
Ptms7	21	0.738	0.736	ns
Ptms9	16	0.483	0.490	ns
Ptms10	18	0.583	0.628	ns
Ptms33	12	0.883	0.747	**
Ptms40	12	0.521	0.438	ns
Ptms41	25	0.713	0.780	**
Ptms42	13	0.721	0.661	ns
Ptms45	13	0.846	0.702	*
Mean/Total	15.6	0.669	0.656	ns

Abbreviations: Na, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; and HWE, Hardy-Weinberg equilibrium, ns = not significant, * $p < 0.05$, ** $p < 0.01$.

3.2. The Total Genetic Diversity and Differentiation of Three Population Classes of *P. chinensis*

The comprehensive analysis of multiple indicators in the six populations of *P. chinensis* showed that the Na and Ne in the populations of class A and class B were statistically higher than in the group C population. There were no statistical difference between class A and class B populations ($p > 0.05$) (Table 3). After the rarefaction process, which eliminated the effects of sample size, the AR and the rare allelic richness in the populations of class A and class B were still higher than in the group C population. Lower values of He and Ho were also found in the class C population. The inbreeding coefficient (F_{IS}) is less than zero in the class A1 and class B populations, while it is greater than zero, and significantly higher than random expectations ($p < 0.05$), in population C, suggesting that heterozygote deficiencies exist in population C.

The general genetic differentiation coefficient, F_{ST} , value of six populations of *P. chinensis* was 0.113 ($p = 0.01$), showing significant structuring. Individual F_{ST} values between populations ranged from 0.027 to 0.178, and all showed significant values ($p < 0.05$) after applying a sequential Bonferroni correction.

Table 3. Genetic diversity of three population classes (A, B, and C) of *Pistacia chinensis*.

Population	Na	Ne	AR	PAR	Ho	He	F_{IS}
Pop A1	7.889	3.879	5.57	0.77	0.769	0.725	−0.048
Pop A2	6.333	3.670	5.15	0.88	0.600	0.604	0.033
Pop B1	7.556	3.937	5.56	1.12	0.725	0.707	−0.013
Pop B2	7.556	4.752	6.23	3.15	0.889	0.746	−0.166
Pop C1	4.889	2.849	3.74	0.16	0.519	0.581	0.118 *
Pop C2	4.111	2.872	3.78	0.25	0.511	0.570	0.129 *

Abbreviations: Na, number of different alleles; Ne, number of effective alleles; AR, allelic richness (standardized for 30 individuals); PAR, rare allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS} , within-population inbreeding coefficient. Asterisks mark values that are significantly higher than those found under random conditions ($p < 0.05$).

3.3. The Genetic Diversity and Differentiation of Six Pre- and Post-Fragmentation Populations

Based on the plant basal diameter or DBH, the three large populations (A1, B1, and C1) of *P. chinensis* were divided into six pre- and post-fragmentation populations. The statistical analysis revealed that the Ne and the AR varied from 2.673 to 4.091 and 3.69 to 5.75, respectively (Table 4). The average Ne in pre- and post-fragmented populations of 3.637 and 3.326, respectively, showed no significant difference ($p = 0.203$), and the average AR, measured at 5.07 and 4.82, respectively, also showed no significant difference ($p = 0.122$). The mean Ho of 0.698 and 0.648 showed no significant difference ($p = 0.160$) in pre- and post-fragmentation populations, respectively, but the average He of 0.682 and 0.639, respectively, were significantly different ($p = 0.009$). The rare allelic richness changed

from 0.19 to 0.44, and the average values among pre- and post-fragmentation populations of 0.37 and 0.30, respectively, showed a significant difference ($p = 0.026$). Whether before or after fragmentation, the inbreeding coefficient of the four populations from populations A1 and B1 were less than zero, while the pre- and post- C1 populations' coefficients were greater than zero and the C1 post-population was significantly different.

Table 4. Three large *Pistacia chinensis* populations divided into six pre- and post-fragmentation populations showing genetic diversity.

Population	Ne	AR	PAR	Ho	He	F _{IS}
PreA1	4.091	5.75	0.44	0.783	0.740	−0.033
Post A1	3.452	5.31	0.35	0.756	0.689	−0.071
Pre B1	3.947	5.67	0.40	0.744	0.716	−0.014
Post B1	3.854	5.46	0.35	0.717	0.679	−0.029
Pre C1	2.874	3.80	0.27	0.567	0.591	0.066
Post C1	2.673	3.69	0.19	0.472	0.549	0.166 *

Abbreviations: Ne, number of effective alleles; AR, allelic richness (standardized for 20 individuals); PAR, rare allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, within-population inbreeding coefficient.
* $p < 0.05$.

The stochastic analysis through 9999 random permutation showed that the total genetic differentiation coefficient F_{ST} of the six subpopulations was 0.086 and was significantly different ($p = 0.010$). The F_{ST} of pre- and post-fragmented populations in the same large population did not show significant differences ($p > 0.05$), and the gene flow also was relatively larger. However, each of the two populations from different large populations displayed significant differences ($p < 0.05$), with a smaller gene flow (Table 5).

Table 5. Population N_M values based on F_{ST} values shown above the diagonal and pairwise F_{ST} values shown below the diagonal for the six *Pistacia chinensis* populations.

	Pre A1	Post A1	Pre B1	PostB1	Pre C1	Post C1
PreA1	0.000	45.385	9.563	7.668	1.932	1.786
Post A1	0.005	0.000	6.102	8.785	2.099	1.920
Pre B1	0.025	0.039	0.000	24.509	1.176	1.132
Post B1	0.032	0.028	0.010	0.000	1.505	1.483
Pre C1	0.115	0.106	0.175	0.142	0.000	29.822
Post C1	0.123	0.115	0.181	0.144	0.008	0.000

Note: Values in bold above the diagonal showed larger gene flows and those in bold below the diagonal were not significantly different from zero.

3.4. The Genetic Diversity and Differentiation of Six Male and Female Populations

After the fragmentation of the landscape pattern in the TIL region, the small populations of *P. chinensis* showed biased sex ratios, while the mainland population had a stable 1:1 sex ratio. Therefore, we divided the three large populations into six populations based on gender, and analyzed whether fragmentation changed the genetic diversity of the male and female populations. Statistics found that the AR of the male populations was slightly less than those of the female populations in A and B, while the male numbers were larger in population C. A significance test showed that male and female populations had no significant differences in average AR (4.94 and 4.96, respectively; $p = 0.810$). For the rare allelic richness, all three male populations were lower than the female populations, and the average rare alleles showed a significant difference (0.38 and 0.46, respectively; $p = 0.024$). Similarly, the three male populations had lower Ho and He values than the females. The average He of male and female groups were not significantly different at 0.669 and 0.687, respectively ($p = 0.356$), but the mean Ho was significantly different (0.655 and 0.697, respectively; $p = 0.041$). An analysis of inbreeding

parameters found that the inbreeding coefficient of males and females within populations A and B were less than zero, but greater than zero in population C, and that the value was larger in the male group than in the female group ($M > F$: $0.127 > 0.011$). The inbreeding coefficient of males in populations A and B were also greater than those in the female populations (Table 6).

Table 6. Three large *Pistacia chinensis* populations divided into six populations based on gender and their genetic diversity.

Population	AR	PAR	Ho	He	F _{IS}
A _M	5.50	0.49	0.746	0.719	−0.034
A _F	5.67	0.55	0.795	0.721	−0.105
B _M	5.59	0.43	0.716	0.716	−0.005
B _F	5.43	0.53	0.741	0.766	−0.114
C _M	3.72	0.22	0.502	0.571	0.127
C _F	3.79	0.29	0.556	0.575	0.011

Abbreviations: AR, allelic richness (standardized for 20 individuals); PAR, rare allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, within-population inbreeding coefficient.

4. Discussion

Landscape fragmentation has affected the GD of *P. chinensis* populations, and some indices reached a significant level. Among the six selected *P. chinensis* populations in this study, the mainland A class population and the B class population, which were not fragmented, had higher levels of GD than the fragmented C class population in the NA, AR, PAR, H_E , and H_O (Table 3), but not at significant levels. Landscape fragmentation decreased or even blocked the gene flow among residual populations [31–35]. Although *P. chinensis* belongs to the wind-pollinated plants, Lu et al. [36] pointed out that the dispersal distance of *P. chinensis* pollen is only about 400 m compared with other anemophilous plants that can spread hundreds of kilometers. Also, Yu and Lu [9] found that the average weight of a *P. chinensis* seed is 0.042 g. Because the fruit tastes slightly bitter, the seed is not easily dispersed by birds or small rodents in the TIL reservoir. Therefore, seeds of *P. chinensis* are dispersed mainly by gravity. In this study, the populations were separated by hundreds to thousands of meters and the N_M among populations may encounter obstacles. Using the genetic differentiation coefficient F_{ST} analysis, we found that there are significant genetic differences among the populations, and using F_{ST} to estimate the gene flow, we found that the gene flow among populations was between 1.202 and 9.072. The maximum value, 9.072, occurred between the B1 and B2 populations, with the other gene flow values between populations being less than 4. This may be because the distance between the B1 and B2 populations is only 302 m, while the distances between other populations are more than 450 m. Furthermore, an analysis of the abiotic factors in the TIL region found that the formation of a regional climate (wind speed, wind direction, temperature, and humidity) during the *P. chinensis* pollination season, and the corresponding geographic landscape elements, may affect the gene flow and form barriers [33,37–39].

By dividing *P. chinensis* populations into pre- and post- populations using the plant rhizosphere, and then performing statistical analyses, we showed that: (1) Pre- populations are slightly higher than post- populations in Na, AR, He, and Ho, but have not yet reached significant levels, and (2) there is a significant difference in the PAR between pre- and post-fragmentation populations. Genetic drift caused by population fragmentation is usually thought to lead first to the loss of rare alleles, but rare alleles are very important for the adaptability of plant populations to continual environmental changes [40]. In this study, compared with pre-populations, post-populations have, to some extent, missing rare alleles, which indicated that the fragmented habitat caused a reduction in the offspring's genetic diversity and that a persistently broken habitat will further affect the viability of all populations. A statistical analysis of the genetic differentiation coefficient found that the total F_{ST} values showed significant differences among the six populations, but that the F_{ST} values of the three pairs of populations, which within the same group are small and not significant, are significant, and the gene flow is also relatively large. Other pairwise comparisons among groups that did not belong to the same population displayed

significant differences and only a small level of gene flow (Table 5), demonstrating that isolation by distance played a key role in hampering the gene flow [41–43].

Because of the reservoir's formation, a large and continuous population was broken into multiple small and isolated residual fragments. The reduction of habitat area directly resulted in the reduction of the number of individuals of the dioecious plant *P. chinensis*, which in turn further caused imbalances in the percentages of male and female individuals and their uneven distribution. Yu and Lu [9] also have discovered that an increased patch size and decreased isolation maintains the sex ratio at ~1:1. Otherwise, the population becomes biased toward males. By analyzing the GD of male and female populations, we found that female populations were not only higher than male populations in AR, PAR, He, and Ho, but that PAR and Ho were significantly different. This may be a result of differences in the utilization and investment of resources by dioecious plants. When under the same environmental stresses, the male and female plants showed different reactions that may accelerate changes in GD [44]. The reduction of GD in the male population will reduce adaptability to the changing environment, while the reduction of individual numbers in the female population will trigger a further population decline.

O'Connell et al. [45] also provided strong evidence that extensive long-distance pollen dispersal plays a primary role in maintaining low genetic differentiation among natural populations of *P. glauca* Voss and helps maintain genetic diversity and minimize inbreeding in small stands in a fragmented landscape.

By studying *P. chinensis* populations in the highly-fragmented landscape in the Thousand-Island Lake region, we demonstrated the negative effects of fragmentation on its genetic diversity. Therefore, the wild *P. chinensis* populations in the TIL region may need some extra measures, such as foreign artificial pollination and seedling transplantation, to maintain its genetic diversity and viability.

Therefore, we believe that there are several important suggestions for the protection of the *Pistacia chinensis* population and dioecious plants in the future:

(1) It is very important to ensure the number of individuals in dioecious plant population, which can promote sufficient pollen flow in the populations. The number of individuals in the population can be increased by collecting seeds, cultivating seedlings, and introducing the seedlings into populations; (2) balancing the proportion of males and females in dioecious plant population and the distribution of individuals; (3) paying attention to the dynamic changes of population number and structure; (4) connectivity between protected species populations can maintain a certain gene flow, maintain their genetic diversity, and guarantee the survival of the population; (5) Because of global climate change, we should focus on the effects of global warming on the reproductive effects of *Pistacia chinensis* and dioecious plants.

5. Conclusions

The study revealed that the GD of the *P. chinensis* population as a whole is relatively high in the Thousand-Island Lake (TIL) region, but its fragmented landscape still led to some loss of rare alleles, especially in a fragmented small population, a post fragmented population, and a male population. The interesting finding is female populations have higher GD than male populations, despite the fact that male populations are generally larger than female ones.

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