Effects of Habitat Fragmentation on the Genetic Structure of Xinjiang Wild Apple (Malus sieversii) Populations (Postprint)

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Abstract

Habitat fragmentation exerts complex genetic effects on plant populations. Within geographic populations, Malus sieversii (wild apple) exhibits multiple fragmented subpopulations. This study selected the Gongliu population, which possesses relatively high genetic diversity, as the research subject, and utilized microsatellite (SSR) markers to genotype 101 plant samples collected from five subpopulation sampling sites. By analyzing differences in genetic diversity and phylogenetic relationships among the subpopulations, we investigated the impact of habitat fragmentation on the genetic structure of Malus sieversii populations. The results demonstrated that genetic diversity differences exist among the five subpopulations, with the two edge-located subpopulations, Saha and Xiao Mohe' er, maintaining high levels of genetic diversity. Due to geographic isolation effects, these five subpopulations can be divided into two distinct genetic lineages, eastern and western. It is recommended that future conservation of Malus sieversii germplasm resources and construction of core collections should prioritize these edge subpopulations and different genetic lineages.

Full Text

Effects of Habitat Fragmentation on the Population Genetic Structure of *Malus sieversii*

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Abstract: Habitat fragmentation has complex genetic effects on plant populations. *Malus sieversii* exists in a few small, fragmented subpopulations within a geographical population. For this study, we selected the geographical population of Gongliu, which has a high level of genetic diversity. The genotypes of 101 individuals from five subpopulations were marked using SSR loci. By analyzing the difference in genetic diversity and the genetic relationships among these five subpopulations, we aimed to investigate the effect of habitat fragmentation on the population genetic structure of *Malus sieversii*. The results showed that different levels of genetic diversity existed among the five subpopulations, in which a high level of genetic diversity was shown in marginal subpopulations of Sahaa and Xiaomoheer. These five subpopulations were clustered into two genetic lineages, which displayed an east-west split due to the effect of geographical isolation. It is proposed that these marginal subpopulations and different genetic lineages should be considered during the processing of germplasm resource protection and the core collection of germplasm for *M. sieversii*.

Keywords: *Malus sieversii*; habitat fragmentation; germplasm resource; genetic structure; marginal subpopulations; geographical isolation; Gongliu County; Xinjiang

1 Materials and Methods

1.1 Sample Collection

For this study, we collected samples from five fragmented subpopulations of *Malus sieversii* in Gongliu County, Xinjiang (Figure 1, Table 1). A total of 101 individual trees were sampled, with spacing between individuals exceeding 50 meters to avoid collecting closely related individuals. Geographic coordinates were recorded for each sample using GPS. The sample distribution included: 18 individuals from subpopulation A (Sahaa), 19 from subpopulation B (Xiaomoheer), 36 from subpopulation C (Kuerdening), 10 from subpopulation D (Axi 1), and 18 from subpopulation E (Axi 2).

1.2 DNA Extraction and SSR Amplification

Total genomic DNA was extracted from fresh leaf tissue (approximately 50 mg per sample) using the CTAB method [**?**]. Ten polymorphic SSR primer pairs previously developed for *Malus* species were selected from the literature [**?**, **?**, **?**]. PCR amplification was performed following the protocols described in these references. Amplified products were separated on 6% polyacrylamide gels and visualized using silver staining. Allele sizes were determined using a DNA ladder standard.

1.3 Data Analysis

Genetic diversity parameters were calculated for each subpopulation, including: number of alleles per locus (Na), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe = 2N/(2N-1) × He), and inbreeding coefficient (F = 1 - (Ho/He)). These calculations were performed using GenAlEx 6.5 [?] and SPSS software. Deviations from Hardy-Weinberg equilibrium were tested using Arlequin 3.11 [?].

To examine spatial genetic structure, isolation by distance (IBD) analysis was conducted by correlating pairwise genetic distances (FST) with geographic distances among subpopulations. Spatial autocorrelation analysis was performed using GenAlEx to test for non-random distribution of genotypes across the sampling area. The significance of IBD was assessed using Mantel tests with 999 permutations.

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2 Results

Genetic diversity varied significantly among the five subpopulations (Table 2, Table 3). The highest levels of genetic diversity were observed in the marginal subpopulations Sahaa and Xiaomoheer, as evidenced by their higher values of Na, He, and uHe compared to the central subpopulations. The overall genetic differentiation among subpopulations was moderate (FST = 0.112, $P < 0.001$).

Clustering analysis revealed two distinct genetic lineages corresponding to an east-west geographic split (Figure 2). The eastern group comprised subpopulations A (Sahaa) and B (Xiaomoheer), while the western group included subpopulations C (Kuerdening), D (Axi 1), and E (Axi 2). This pattern was supported by both neighbor-joining tree analysis and STRUCTURE results (K = 2).

Isolation by distance analysis showed a significant positive correlation between genetic and geographic distances ($r = 0.68$, $P = 0.032$), indicating that geographic isolation contributes to genetic differentiation among subpopulations. However, the IBD pattern was primarily driven by the differentiation between the two major genetic lineages rather than by gradual isolation across the entire sampling range.

[Figure 5: see original paper]

Fig. 5 Correlation analysis of pairwise genetic distances and geographical distances among five fragmented sampling locations

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3 Discussion

Habitat fragmentation has profoundly impacted the genetic structure of *Malus sieversii* populations in Gongliu County. The detection of two genetic lineages with an east-west split suggests that historical geographic barriers have shaped the current population structure, while recent fragmentation has further isolated these subpopulations. The higher genetic diversity observed in marginal subpopulations contrasts with the typical expectation that central populations maintain higher diversity, possibly indicating that these marginal areas serve as refugia or have experienced different demographic histories.

The significant isolation by distance pattern underscores the importance of gene flow limitation in maintaining genetic differentiation among subpopulations. However, the discrete clustering into two lineages rather than a continuous IBD pattern suggests that major geographic features (likely mountain ranges or river systems) have created substantial barriers to gene flow, overriding the effects of simple geographic distance.

For germplasm conservation, these results highlight the need to preserve representatives from both genetic lineages, particularly the genetically diverse marginal subpopulations. The subpopulations at Sahaa and Xiaomoheer should be prioritized for in situ conservation due to their high genetic diversity and unique genetic composition. Ex situ collection strategies should ensure sampling from both eastern and western lineages to capture the full genetic diversity of *M. sieversii* in this region.

Future studies should investigate the adaptive significance of the genetic differences between lineages and monitor temporal changes in genetic diversity as fragmentation continues. The integration of genomic approaches and ecological niche modeling would provide deeper insights into the evolutionary history and future persistence of this important wild apple resource.

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Note: Figure translations are in progress. See original paper for figures.