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Genetic Diversity, Mating System, and Seed Viability Reveal a Trade-Off between Outcrossing and Inbreeding in *Pinus yunnanensis* var. *tenuifolia*, an Ecologically Important Conifer Species Growing in a Hot-Dry River Basin Habitat in Southwest China

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Abstract: Revealing the relationship between the mating system (i.e., the outcrossing/inbreeding degree) and the fitness of seeds in tree species under wild conditions is essential for understanding the ecological adaptability and evolutionary stability of the species. This study collected open-pollinated seeds from seven wild populations of *Pinus yunnanensis* var. *tenuifolia* that exhibited fragmentation in the Nanpan–Hongshui River basin, an ecologically fragile area in China. The seeds and sprouts (germinated seeds) from 20 families were genotyped (24 seeds and 24 sprouts per family) using twelve microsatellite loci to reveal the genetic diversity, mating status, and effect of inbreeding on the three seed quality indicators (thousand-seed weight, germination rate, and germination potential). The three seed quality indicators differed significantly between families ($p < 0.001$). Higher values of genetic diversity (except the observed heterozygosity) were observed in the sprout group than those in the seed group. Families from different populations showed a notable genetic differentiation (Φst = 0.12), and a large part of families from the common populations had a high degree of coancestry, which signified that the current habitat fragmentation is limiting gene flow between populations. High levels of outcrossing rates (tm) were observed in both the seed group ($t_m = 0.974$) and the sprout group ($t_m = 0.978$), indicating that a low proportion of seeds were self-fertilized. Although there was a slightly higher single outcrossing rate ($t_s = 0.888$) and a lower proportion of biparental inbreeding ($t_m - t_s = 0.077$) in sprouts compared to the seeds ($t_s = 0.871$, $t_m - t_s = 0.091$), indicating that a part of inbred seeds were purged during the germination stage, curve fitting between the outcrossing rate and seed quality indicators showed that a certain degree of biparental inbreeding (t_s between 0.89 and 0.91 and $t_m - t_s$ between 0.09 and 0.11) did have a positive effect on seed germination ability. This highlights that excessive inbreeding or outbreeding seems to be unfavorable to seed viability. The peculiar relationship between seed viability and the mating system in *P. yunnanensis* var. *tenuifolia* was likely an evolutionary consequence of a trade-off between the nature of mixed mating and its specific ecological niche.

Keywords: genetic differentiation; habitat fragmentation; mating system; *Pinus yunnanensis* var. *tenuifolia*; reproduction fitness

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1. Introduction

Many tree species in the wild are subjected to varying degrees of habitat fragmentation. This fragmentation directly results in a reduction in population size and isolation between populations. Pollen gene flow is restricted in fragmented habitats, leading to an increase in inbreeding rates (i.e., selfing and mating among relatives) and a decrease in genetic diversity [1–5]. Self-pollination and mating among relatives can reduce phenotypic fitness, physiological efficiency, and offspring viability in natural outcrossing species [6,7]. These

effects are known as inbreeding depression (ID). However, controversial results have been reported in some species, suggesting that inbreeding may be beneficial for the species' survival in special habitats, such as marginal and isolated environments [8–12].

Conifers are typical species with mixed selfing and outcrossing, and its ID prevalently occurs at the stages of seed production of the parent, seed development and germination, juvenile survival, growth, and fertilization [8]. In conifers where polyembryony is common, ID is first manifested as a decrease in the ratio of full seeds in cones and a sharp decline in seed yield [13–15], and then, as a decrease in individual viability, growth, and fecundity in offspring stands [16,17]. These results are mainly derived from widely and continuously distributed species. For species that have long evolved under distinct habitats (such as the evolution of a distinct variety from the marginal population), the association between the inbreeding degree of parental populations and the fitness of progeny is still unclear, but it is essential to understand the mating system evolution in plant species.

Pinus yunnanensis var. *tenuifolia* (PYT) is a geographical variety of *Pinus yunnanensis* that migrated eastward from the warm and wet Yunnan Plateau in the Middle subtropical zone to adapt to the dry and hot valley habitat in the South subtropical zone [18–20]. It is mainly distributed along the Nanpan River and Hongshui River basins at the junction of Yunnan, Guizhou, and Guangxi provinces at an altitude of 300–1300 m. This growth region of PYT belongs to the typical carst landform with relatively weak ecological stability in China. As a predominant tree species in this area, PYT plays an important role in maintaining the ecological stability of the forest community. Meanwhile, it is an important timber and rosin production tree and occupies an irreplaceable position in the forest resources of the Nanpan–Hongshui River basin [21,22]. Unfortunately, most wild populations of PYT have eroded to small patches in past decades due to the invasion of fast-growing exotic tree species (eucalypts) and other economic trees (such as chestnuts and oil tea); thus, the preservation of genetic resources for this tree species is urgent. Recently, the population structure, the geographical variation in the cone and seed characteristics of PYT were studied for its stand protection and resource management [23–25]. However, few studies focus on its current mating status and genetic effect on its offspring, which is essential for evaluating its future genetic potential and ecological sustainability.

Here, the open-pollinated seeds from seven wild PYT populations were collected, and the seed quality traits, including thousand seed weight (TSW), germination rate (GR), and germination potential (GP), were assessed. The seeds and sprouts (germinated seeds) of twenty families were genotyped using twelve microsatellite markers to analyze (1) the current genetic diversity and mating system (selfing or outcrossing rate) of natural populations of PYT under the process of habitat fragmentation and to assess (2) whether the mating system has a significant effect on the seed's viability? The results will provide enlightenment for the conservation and management of PYT genetic resources.

2. Materials and Methods

2.1. Plant Sample Collection

Seven representative wild populations of PYT, which were previously reported in Bai et al. [23], were selected for this study. These populations are located in the Nanpan–Hongshui River basin (see Table S1). A total of 20 healthy adult trees with productive cones were randomly sampled from these populations as mother trees for cone collection. The diameter at breast height (DBH) and geographical coordinates of each tree were recorded. No less than thirty mature cones were randomly collected from the upper crown of each mother tree in mid-November 2021, put into labeled cloth bags, and brought back to the laboratory for further study. All voucher specimens were preserved in the Forestry College of Guangxi University.

2.2. Seed Quality Measurement

The cones were exposed to sunlight from three to seven days, and the shed seeds were collected and de-winged. Four replicates of 100 seeds per mother tree were selected and sterilized with a 0.05% potassium permanganate solution for 2 h. After rinsing in

distilled water several times, the seeds were evenly laid in germination boxes lined with two sheets of filter paper, moistened with deionized water, and then placed in an incubator. The incubator programs alternated between daytime (12 h) and nighttime (12 h). The nighttime temperature was set at 20 ± 1 °C with an illumination intensity of 0 Lux, while the daytime temperature was 24 ± 1 °C with 1500 Lux. Daily observations and recordings were conducted to calculate the germination rate (GR) and germination potential (GP) in order to assess the seed quality of the mother tree. The germination testing lasted 31 days, from 7 June 2021 to 8 July 2021. GR and GP were calculated according to the following formulas [26]:

$$\text{GR (\%)} = (\text{number of seeds germinated}/\text{total number of seeds tested}) \times 100$$

$$\text{GP (\%)} = (\text{number of seeds germinated when germination reached its peak}/\text{total number of seeds tested}) \times 100$$

After germination, the germinated seeds of each family were collected into 1.5 mL PE tubes and stored at −80 °C for genotyping.

2.3. DNA Extraction and Genotyping

A total of 480 seeds (without conducting the germination test) and 480 sprouts (germinated seeds) from 20 families (24 seeds plus 24 sprouts per family) were used for DNA extraction. The DNA in each seed and sprout was isolated according to the SDS [27,28] and CTAB methods [29], respectively. Twelve fluorescently-labeled (FAM and TAMRA) microsatellite markers [30–33] were selected for genotyping (see Table S2). PCR was performed in a total volume of 10 µL containing 50 ng of DNA, 10 mM 10 × buffer (containing Mg^{2+}) and 0.25 mM dNTPs, forward and reverse primers at 0.3 µM each, and 0.5 U of Taq polymerase (Takara Biomedical Technology Co., Ltd., Beijing, China); ultrapure water was added to bring the total volume to 10 µL. All amplification reactions were performed in a Biometra TGradient PCR 96 instrument (Analytik Jena, Jena, Jena, Germany) according to the following program: 94 °C for 3 min; 32–34 cycles of 94 °C for 30 s; Tm of respective primer pair for 30 s; 30 s at 72 °C; and a final extension at 72 °C for 10 min, followed by storage at 4 °C. All microsatellite loci were amplified independently with 12 separate PCRs for each sample. The PCR products were separated by using the DNA sequencer ABI3730 XL (Applied Biosystems, Foster City, CA, USA) with a ROX-500 internal size standard, and allele sizes were scored using ABI GENEMAPPER v4.0. The output profiles were checked to confirm the allelic size.

2.4. Data Analysis

Nested analysis of variance (ANOVA) was conducted on three seed quality indicators (TSW, GR, and GP) between populations and families within the population. The GR and GP values were transformed to arcsine square root values before analysis to achieve a normal error distribution (See Figure S1) [34]. Spearman's correlation between three seed quality indicators were estimated. All analyses were performed using R software version 4.3.0 [35].

Family-level genetic diversity parameters, including the observed number of alleles (Aobs), allelic richness (Arich), observed heterozygosity (Hobs), expected heterozygosity (Hs), Shannon's diversity index (SH), and Wright's fixation index (F), for seeds and sprouts were calculated using the R package hierfstat [36]. Analysis of molecular variance (AMOVA) was conducted using poppr [37] with a hierarchical formula (population/family/individual/within individual) [38,39]. The coancestry between individuals was estimated using the snmf [40], a non-negative matrix decomposition algorithm in the R package LEA [41]. The optimal number of ancestors (K value) was determined based on the minimum cross-entropy [40]. The Ward error sum of squares hierarchical clustering (ward.D2) method was performed among families [42], and the optimal number of clusters was determined using the elbow method [43]. An R package, ggtree, was used to visualize the cluster tree [44]. Isolation by distance (IBD) among families was tested using the mantal test between a matrix of family-level genetic distances and geographic distances [45].

To investigate the impact of the mating system on seed quality, we compared the family-level mating system parameters between seed and sprout groups. The mating system analysis was conducted using the MTLR 3.4 software [46,47] under mixed mating systems. The estimated parameters included the multilocus outcrossing rates (t_m), single-locus outcrossing rates (t_s), mating among relatives ($t_m - t_s$), the proportion of full-sib ($r_{p\ (m)}$), the effective number of pollen donors (N_{ep}) = $1/r_{p(m)}$, and the proportion of half-sib (P_{hs}) =$1 - r_{p(m)}$. All parameters were estimated using the expectation–maximization (EM) algorithm. The standard error of these estimators was assessed using 1000 bootstraps. Linear and quadratic regressions were applied to the seed quality indicators (TSW, GR, and GP) in relation to the mating system parameters (t_m, t_s, and $t_m - t_s$).

The differences between the seed and sprout groups in the genetic diversity parameters, variance components, and mating system parameters were detected using a Monte Carlo permutation test instead of parametric test methods, which is inappropriate for molecular data [48]. The Monte Carlo resampling times were 9999, 999, and 9999, respectively.

3. Results

3.1. Seed Quality

The thousand-seed weight (TSW), germination rate (GR), and germination potential (GP) varied significantly among populations and families (see Figure S2). The mean TSW, GR, and GP among families were 14.48 g (9.00–19.60 g), 40.14% (10.60%–82.25%), and 12.39% (4.58%–27.40%), respectively. The correlation between TSW and GR or GP was insignificant, while a significant correlation between GR and GP was detected (see Figure S3).

3.2. Genetic Diversity

The offspring (seeds and sprouts) of PYT maintained moderate genetic diversity at the family level (Table 1). When comparing the seed and sprout groups, more than half of the families in sprouts exhibited higher values in Aobs (16 out of 20), Arich (16 out of 20), Hs (12 out of 20), and SH (13 out of 20), but lower values in Hobs (14 out of 20). Furthermore, the mean values of genetic diversity parameters (Aobs, Arich, Hs, and SH) in the sprout group were significantly higher than those in the seed group. However, the observed heterozygosity (Hobs) in the seed group was higher than that in the sprout group, thus leading to the Wright's fixation index (F) being higher in sprouts (0.113) than in seeds (0.044) (Figure S4).

Table 1. Genetic diversity parameters of twenty *P. yunnanensis* var. *tenuifolia* families (seeds/sprouts).

Family ID	Sample Size	Aobs	Arich	Hobs	Hs	SH	F
BY-24	24/24	3.8/4.0	3.6/3.8	0.528/0.496	0.477/0.472	0.838/0.826	−0.070/0.003
BY-23	24/24	3.9/4.2	3.8/4.0	0.497/0.433	0.440/0.503	0.801/0.912	−0.125/0.174
BY-37	24/24	4.3/5.5	4.2/5.3	0.479/0.336	0.506/0.547	0.926/1.076	0.093/0.369
BY-47	24/24	5.0/6.1	4.8/5.8	0.496/0.465	0.554/0.582	1.057/1.151	0.103/0.220
CJ-45	24/24	5.0/5.3	4.9/5.0	0.604/0.586	0.598/0.552	1.131/1.039	−0.02/−0.028
QX-41	24/24	5.2/6.4	5.0/6.1	0.520/0.556	0.560/0.595	1.079/1.208	0.074/0.058
QX-14	24/24	6.3/5.9	6.0/5.7	0.487/0.506	0.582/0.552	1.186/1.100	0.150/0.057
BW-26	24/24	6.3/6.0	6.0/5.8	0.498/0.560	0.606/0.590	1.219/1.193	0.191/0.049
XQ-1	24/24	4.0/4.8	3.8/4.6	0.455/0.472	0.421/0.480	0.769/0.935	−0.008/0.047
XQ-13	24/24	5.3/6.9	5.1/6.7	0.470/0.401	0.567/0.638	1.100/1.333	0.185/0.335
XQ-34	24/24	3.7/4.2	3.5/4.0	0.542/0.540	0.451/0.518	0.779/0.921	−0.122/−0.044
XQ-7	24/24	4.7/7.0	4.5/6.8	0.502/0.460	0.507/0.637	0.949/1.317	−0.009/0.241
WJ-18	24/24	5.0/5.7	4.8/5.4	0.506/0.482	0.572/0.594	1.081/1.162	0.156/0.213
WJ-24	24/24	4.7/5.5	4.5/5.3	0.368/0.377	0.467/0.538	0.893/1.072	0.195/0.187
WJ-28	24/24	4.9/3.8	4.8/3.7	0.436/0.392	0.505/0.406	0.982/0.732	0.110/0.030
WJ-41	24/24	6.1/5.2	5.9/5.0	0.521/0.500	0.603/0.531	1.223/1.021	0.031/0.025
DT-17	24/24	3.8/3.9	3.7/3.8	0.457/0.394	0.462/0.438	0.817/0.809	0.045/0.194
DT-4	24/24	4.7/5.0	4.5/4.8	0.468/0.478	0.500/0.499	0.948/0.982	0.039/0.044
DT-1	24/24	4.4/4.5	4.2/4.4	0.553/0.470	0.524/0.540	0.937/1.004	−0.046/0.095
DT-40	24/24	4.2/5.3	4.0/5.0	0.521/0.515	0.491/0.545	0.899/1.058	−0.096/−0.013
mean	24/24	4.8/5.3 ***	4.6/5.0 ***	0.495/0.471 **	0.520/0.538 *	0.981/1.043 **	0.044/0.113 ***

Note. Aobs, mean number of alleles observed per locus; Arich, mean allelic richness per locus; SH, Shannon's diversity index; Hobs, observed heterozygosity; Hs, expected heterozygosity; F, Wright's fixation index. The difference between seeds and sprouts were tested by Monte Carlo permutation test (9999 times), and *, **, and *** indicated significant differences at the levels of 0.05, 0.01, and 0.001.

3.3. Genetic Structure

There were obvious genetic differentiations between populations (Φst (Phi) = 0.122/0.102) and between families within populations (0.151/0.151) in seed/sprout groups. All strata of variance components were significantly different between the seed and sprout groups except for that between families within the population (Table 2, Figure S5). The variance component value between populations in the sprouts was lower than that in the seeds, whereas the variance component between individuals within families in sprouts was significantly higher than that in the seeds. The variance component between populations and between families marginally decreased, but that among individuals (seeds) within families significantly increased after germination.

Table 2. AMOVA results of twenty *P. yunnanensis* var. *tenuifolia* families (seeds/sprouts).

Source of Variation	DF	Sum Sq	Mean Sq	Var Comp	Diff Coef (Φst)
Between populations	6	1181.381/1082.009	196.897/180.335	1.034/0.883 **	0.122/0.102
Between families within population	13	785.455/829.850	60.420/63.835	1.121/1.175	0.151/0.151
Between individuals within family	460	3036.305/3428.562	6.601/7.453	0.294/0.833 **	0.047/0.126
Within individual	480	2885.913/2777.849	6.012/5.787	6.012/5.787 **	
Total	959	7889.054/8118.271	8.226/8.465	8.462/8.677 **	

Note. ** the difference in variance components between seed and sprout groups are significantly ($p < 0.01$) unequal to zero based on the 999 times tested using the Monte Carlo permutation test. Φst was the genetic differentiation coefficient [49].

Coancestry analysis showed that the optimal number of ancestries (the elbow point of the minimum cross-entropy) was four in the seed group and five in the sprout group (Figure 1A,B). Hierarchical clusters based on the genetic distance between families also showed consistent results in the coancestry analysis (Figure 1C). Families from the common populations XQ, BW, BY, CJ, and DT exhibited high levels of coancestry (Figure 1D,E), suggesting that gene flow between those populations was relatively limited. The coancestry level was lower in the WJ and QX populations, possibly indicating gene exchange between them and other localities. The seed quality indicators (Figure 1F) varied among families and did not show a clear pattern with the coancestry results.

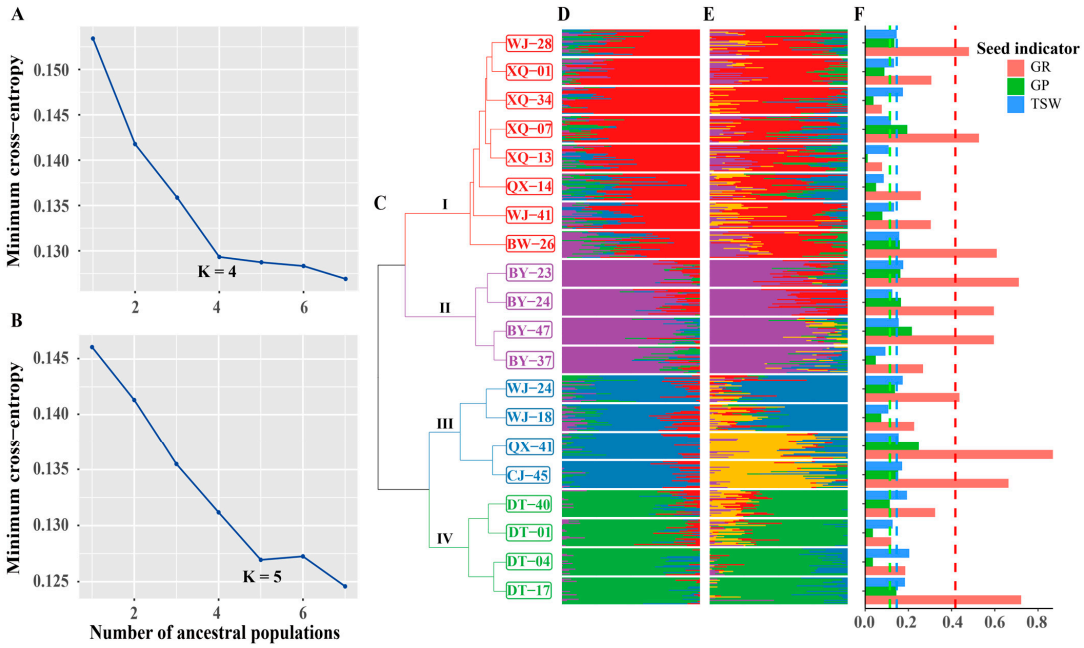


Figure 1. Hierarchical cluster, individual coancestry, and seed quality indicators of twenty *P. yunnanensis* var. *tenuifolia* families. (**A**,**B**), the optimal number of ancestral populations (K value) of seed group (**A**)

and sprout group (**B**) were determined using the minimum cross-entropy values; (**C**) hierarchical clustering (Ward. D2 method) of twenty families combining the seed and sprout genotyping data; (**D**,**E**), the bar plot of ancestral coefficients among 480 seeds (**D**) and 480 sprouts (**E**); (**F**), the bar plot of three seed quality indicators, with each indicator standardized within the range between zero and one.

A significant pattern of isolation by distance (IBD) among families was detected (Figure 2A,B). However, the scatter plot between the geographic and genetic distance showed a slightly discontinuous cloud of points, implying that families from different populations exhibit noticeable differentiation (Figure 2C).

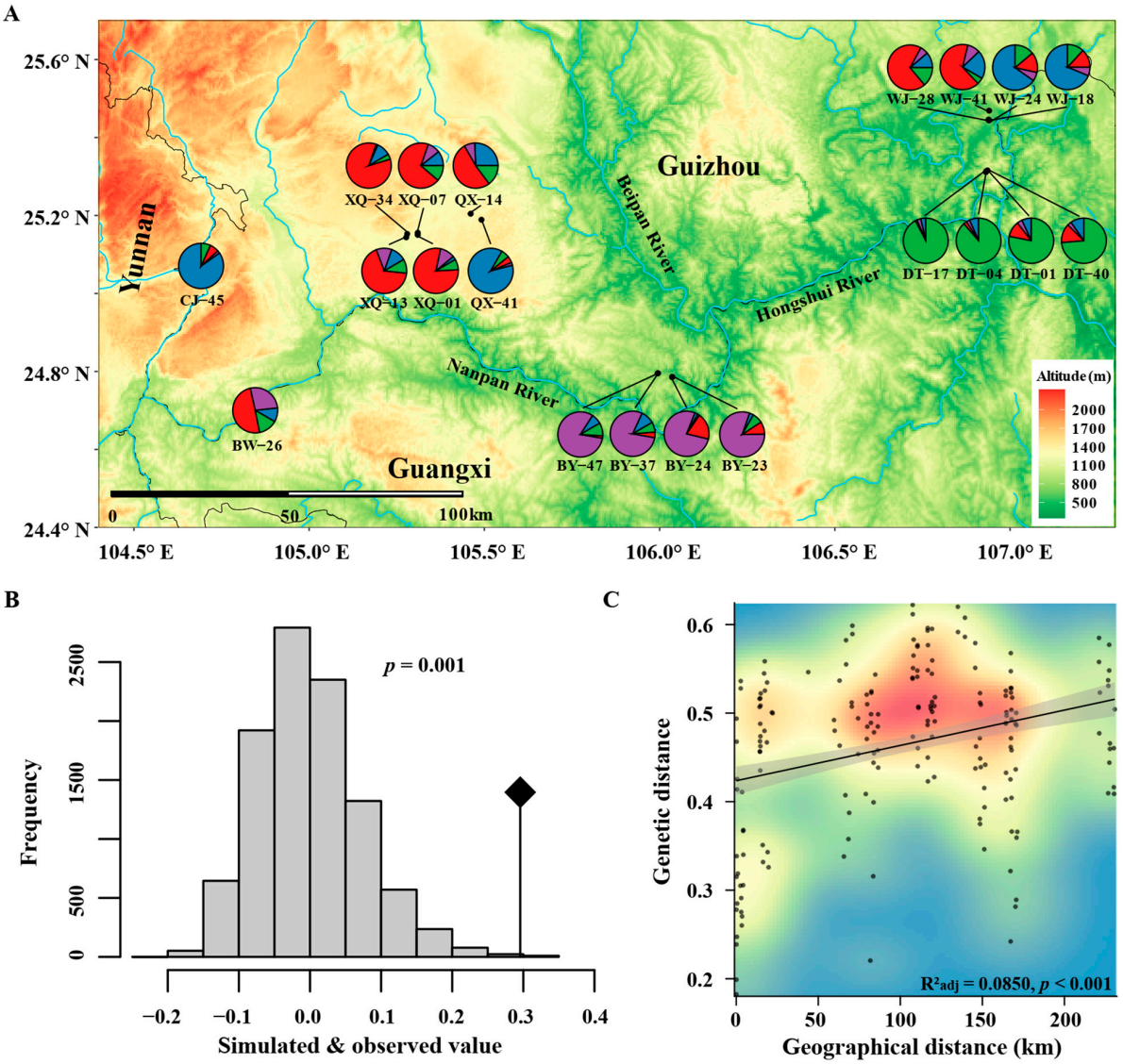


Figure 2. Spatial genetic structure of twenty *P. yunnanensis* var. *tenuifolia* families. (**A**) The geographical distribution pattern of the coancestry coefficient among families; (**B**) the isolation by distance (IBD) tested using the Monte Carlo permutation test (999 times); (**C**) the 2-dimensional kernel density estimation plot shows the relationship between geographical and genetic distance among families.

3.4. Mating System

There were high and comparable multilocus outcrossing rates (t_m) in seeds (t_m = 0.963) and sprouts (t_m = 0.965) of PYT (Figure 3, Table S3), indicating that a few (3.5%–3.7%) seeds were inbred due to self-fertilization. The median of single outcrossing rates (t_s) was marginally higher (p = 0.053) in sprouts than in seeds. The biparental inbreeding ($t_m - t_s$), multilocus correlated paternity ($r_{p(m)}$), effective number of pollen donors (N_{ep}), and the

proportion of the half-sib (P_{hs}) were statistically insignificant between sprouts and seeds. However, the medians of $t_m - t_s$ and $r_{p(m)}$ were slightly lower in sprouts than in seeds, while the N_{ep} and P_{hs} were higher in sprouts than in seeds. In addition, more families in sprouts exhibited higher values for t_s (11 out of 20), N_{ep} (13 out of 20), and P_{hs} (13 out of 20) while showing lower values for $t_m - t_s$ (12 out of 20), and $r_{p(m)}$ (13 out of 20) compared to those in seeds (Figure 4). These results indicated that inbred seeds were partially purged during germination.

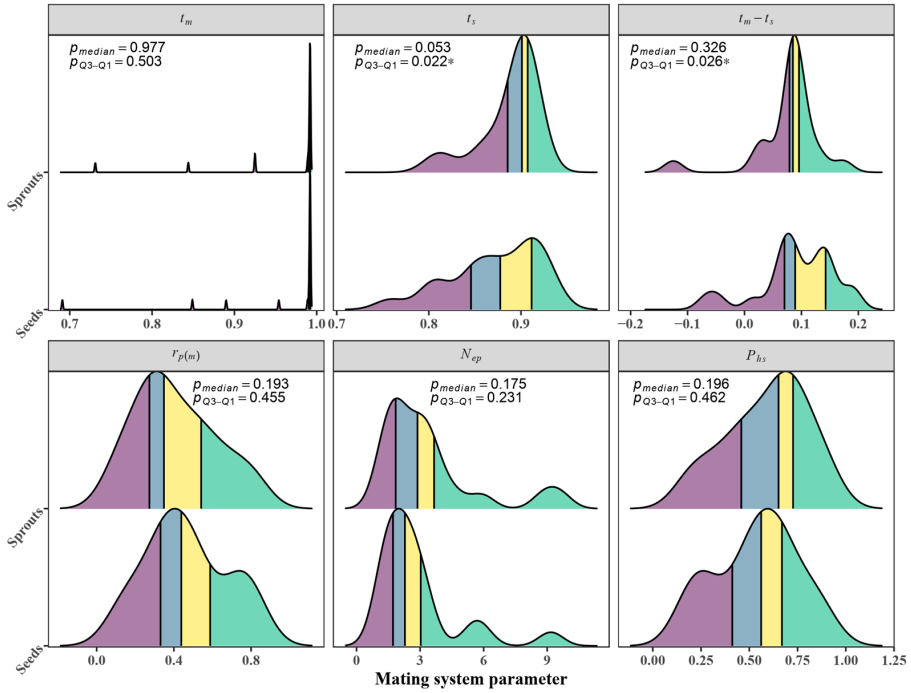


Figure 3. Comparison of family-level mating system parameters between seeds and sprouts of 20 *P. yunnanensis* var. *tenuifolia* families. The vertical lines dividing each density plot into four colored parts, from left to right, represent the lower quartile (25th percentile), the median (50th percentile), and the upper quartile (75th percentile). P_{median} indicated the probability that the observed median between seeds and sprouts is not different. $P_{Q3–Q1}$ indicated that the range between upper and lower quartile was different ($p < 0.05$ denoted by *) or not between sprouts and seeds. The probability was determined using a Monte Carlo permutation test with 9999 iterations.

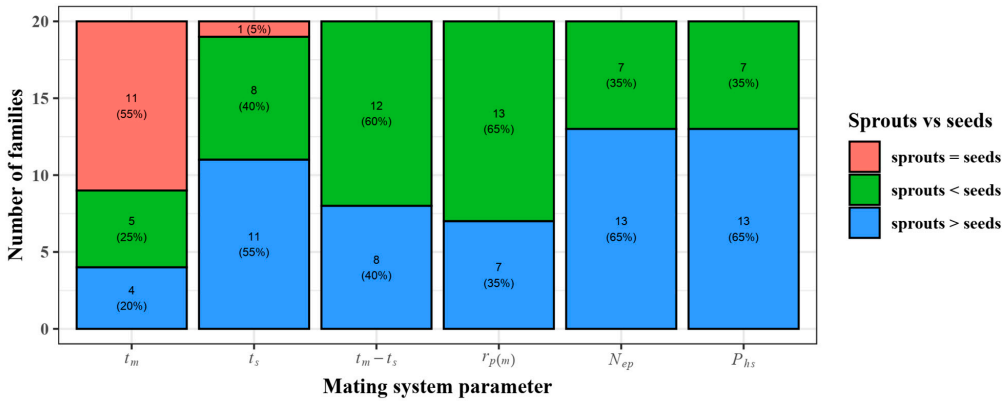


Figure 4. The number and proportion (in parentheses) of families with the different mating system parameters between the seed and sprout groups.

It is worth noting that the distributions of t_s and $t_m - t_s$ in sprouts were more concentrated than in seeds, especially the values between the 25th percentile and 75th percentile

(Figure 3). The suggestion is that the levels of inbreeding among many families became more similar and convergent after the seeds germinated.

3.5. Relationship between the Outcrossing Rate and Seed Quality

Multilocus outcrossing rate (t_m) was negatively correlated with TSW under the linear and quadratic model. No significant correlation was found between tm and GR and GP. Significant correlations were detected between t_s and all three seed quality indicators under the quadratic model. Similar associations were found between $t_m - t_s$ and GR or GP. These results indicated that mixed mating with a certain degree of biparental inbreeding (about 9%–11%) had a positive effect on seed germination ability (Figure 5).

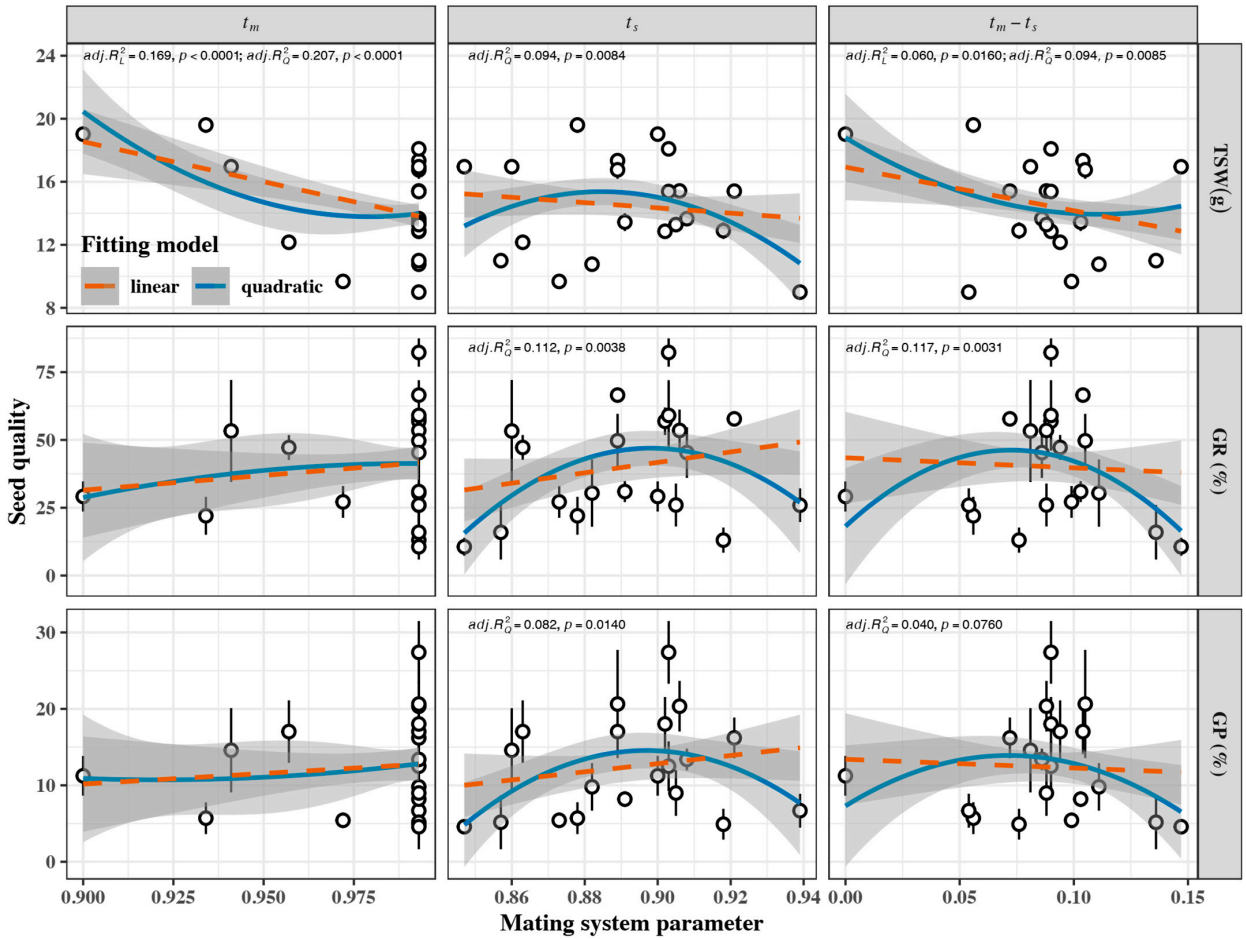


Figure 5. Relationship between inbreeding degree (t_m, t_s, and $t_m - t_s$) and seed quality indicators (TSW, GR, and GP). $adj.R_L^2$ and $adj.R_Q^2$ are the adjusted determination coefficients of linear regression and quadratic regression.

4. Discussion

4.1. Seed Quality

Seed quality plays a vital role in the regeneration of wild stands and the reforestation of artificial plantations. Seed mass and germinability are considered important indicators in evaluating seed quality [50–52]. Our study revealed a significant variation ($p < 0.01$) in seed mass and germinability among populations and families. However, families from common populations did not exhibit similar seed quality, even though more families (3 out of 4) in clusters II (purple) and III (blue) seem to have high values of GR (greater than the overall mean), while more families in cluster IV (green) demonstrate a high value of TSW (Figure 1F). This result indicates that seed quality is likely more affected by the genetic factors (mating patterns and genetic heterogeneity) and microenvironmental variations

among mother trees rather than the population. It supports the viewpoint that the variation between maternal genotypes plays an important role in seed development [50,53,54].

The germination rate of seeds in PTY wild populations was 40.14%, which was lower than the germination rate of the *P. yunnanensis* seeds (GR = 58%), but close to the germination rate of *P. kesiya* var. *langbianensis* in the southern region (43%) [55]. These results are likely related to the unique habitat of PYT. Compared to *P. yunnanensis*, PYT and *P. kesiya* var. *langbianensis* may have undergone more similar habitat selection effects [55]. In addition, the GP measured in this study was significantly lower than the GR, indicating that the seed germination period was not concentrated, and the emergence time was asynchronous. We speculated that it may be influenced by long-term habitat selection (stress) while being affected by the mating system (inbreeding or outcrossing level) of the mother tree. The area where PYT is located is a typical seasonal arid and hot region with frequent foehn winds in spring [18,20,24]. The asynchronous emergence of seedlings may be beneficial for avoiding adverse meteorological conditions and ensuring that some seeds can germinate into seedlings under suitable weather conditions [56]. Some studies suggest that high temperatures promote the seed germination of *P. yunanensis* and *P. densata* [57–59]. It remains to be further explored whether PYT also exhibits similar characteristics. No significant association between seed mass and germination capacity was detected in our study (Figure S3). It was compatible with some species [51,52,60] but inconsistent with others reported [60–63]. It indicates that the effect of seed mass on seed germination capacity is likely species-specific and has little effect on predicting seed germination capacity in PYT.

4.2. Genetic Diversity and Differentiation and Its Causing and Aftereffect

Genetic diversity and genetic differentiation are important indicators for evaluating the sustainable evolution capacity of populations [64,65]. Our results showed that the offsprings of the current wild population of PYT still maintain substantial genetic diversity at the population and family levels. The diversity is comparable to that estimated by the adult trees in wild populations [66]. However, AMOVA and coancestry clustering analysis indicate that genetic differentiation between populations cannot be ignored. Although PYT, like other pine trees, produces saccate pollen that can be transported and pollinated over long distances by wind [67,68], helping to maintain its genetic diversity, the geographic isolation resulting from habitat fragmentation may have hindered gene flow between populations.

Compared to the seeds, the sprouts have higher genetic diversity, lower population genetic differentiation, and higher between-individual differentiation within the family. Undoubtedly, under the premise of unchanged maternal genetic variance, this change can only be attributed to the paternal parent. It means that more heterogenous pollen pollinated offspring are more successful in germination. Strangely, the sprouts also exhibited relatively low observed heterozygosity and a higher fixation index, indicating that some inbred offspring still possessed sufficient germination abilities. In other words, the germination process not only ensures the germination of more heterozygous offspring but also preserves some inbred offspring to some extent. This result indicates that despite the natural population of PYT being subject to varying degrees of natural or human interference, its elimination effect through the seed germination process has, to some extent, maintained a trade-off between outcrossing and inbreeding offspring. It is generally believed that when there are sufficient pollinators, outbreeding is beneficial for masking recessive deleterious and lethal genes, ensuring the viability of offspring [47,69–71]. When there is a shortage of pollinators in the population, inbreeding plays a crucial role in maintaining population stability and sustainability [9,10]. Therefore, the changes in the genetic diversity and differentiation patterns of PYT seeds before and after germination are likely the result of their long-term adaptation to the habitat.

4.3. Relationship between Seed Quality and Mating System and Its Highlights on Adaptive Evolution

Both seeds and sprouts exhibit a high level of outcrossing (t_m (seed) = 0.963, t_m (sprout) = 0.965; t_s (seed) = 0.871, t_s (sprout) = 0.888) in our study. Due to the skewness of the mating system parameters (Figure 3), we used the median instead of the mean to compare the differences between the seed and germination groups. Although no significant statistical differences were detected between the seed and sprout groups in all mating system parameters, the t_s in the sprouts was marginally higher than that in the seeds (P median = 0.053). The mating parameters in the sprouts showed slightly lower biparental inbreeding ($t_m - t_s$) values and full sibling ratio ($r_{p(m)}$), higher effective number of pollen donors (N_{ep}) and half sibling ratios (P_{hs}) compared to the seeds. These results indicate that some inbred seeds are eliminated or filtered out during seed germination. This is consistent with the common inbreeding depression phenomenon exhibited by other conifers [8,72,73]. Some studies have confirmed a negative correlation between seed germinability and its level of inbreeding [14,74]. It is worth noting that the distributions of t_s and $t_m - t_s$ are more convergent after seed germination (Figure 3). The range value between the upper and lower quartiles of the sprouts is significantly smaller than that of the seeds. This means that while inbred offspring are partly eliminated, a few outcrossing offspring are also excluded due to their failure to germinate. This implies that excessive outcrossing may be detrimental to the germination of PYT seeds.

The correlation between the mating system parameter and seed germination capacity further supported the above conclusion. The seed germination rate (GR) and germination potential (GP) exhibited a trend of initially increasing and then decreasing with the increase in t_s and $t_m - t_s$. This suggests that both over-inbreeding and over-outbreeding are detrimental to the germination of PYT seeds. This coincides with the inherent mixed mating pattern of coniferous tree species, which is more advantageous for species survival and coping with abnormal environments (such as insufficient pollen donors due to extreme weather or in marginal habitats) while maintaining the dominance of outcrossing [11,75,76]. As a variant of *Pinus yunnanensis*, PYT has evolved to thrive in hot and dry habitats at the southeastern edge of its natural range. This adaptation allows PYT to benefit from outcrossing, a common advantage among conifers. On the other hand, as the founder population of early occurrence and expansion, the peripheral population of *P. yunnanensis* inevitably faces heterogeneous habitat stress (dry and hot) and insufficient pollen donors. In such a situation, maintaining a certain level of inbreeding is crucial to ensure reproduction and population demography. Therefore, the unique relationship between the mating system and the germination rate of PYT may be linked to its population evolutionary history and ecological niche.

While the thousand-seed weight (TSW) was negatively correlated with $t_m - t_s$, consistent with reports in other species [77], there is also a negative correlation between tm and TSW. However, the correlation between tm and GR or GP is insignificant. We infer that this is mainly due to the serious skewness of the distribution of tm values (Figure 5), which distorted the results of the correlation analysis. Several studies have revealed that the seed mass is mainly influenced by self-fertilization in conifers [8,14,52]. However, our study showed an extremely low level of self-fertilized seeds. We speculate that the self-fertilized seeds may have died at an early stage of seed development [15], thus failing DNA extraction and genotyping. Therefore, the outcrossing rate was likely overestimated in our study, which indirectly resulted in the skewness of t_m.

5. Conclusions and Limitations

This study analyzed the genetic diversity and mating system of PYT wild populations, which are experiencing habitat fragmentation. The current wild populations still exhibit rich genetic diversity in their offspring, but moderate genetic differentiation has occurred among families from different populations. This indicates that habitat fragmentation has had an undeniable negative impact on gene flow among populations. Although habitat

fragmentation may exacerbate inbreeding within the population, PYT purged some inbred and outbred offspring through seed germination, optimizing the proportion of inbred and outcrossing offspring. This trade-off mechanism is likely related to its population occurrence and evolutionary history. As a geographical variant of *P. yunnanensis* evolved at its southeastern edge to adapt to the dry and hot habitat, this mechanism is beneficial for buffering the disturbance caused by edge heterogeneity habitat on its survival and reproduction, thereby maintaining population regeneration and development.

It should be pointed out that this study was based on a seed germination test conducted indoors. This study concluded that excessive outcrossing or inbreeding were not favorable for PYT seed germination. In contrast, seed germination under wild conditions is influenced by various environmental factors. Whether the seed germination of PYT under natural conditions is stimulated or inhibited by specific environmental factors needs further exploration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15060982/s1, Figure S1: The frequency distribution and residual distribution of three see quality indicators; Figure S2: The difference between populations and families in three seed quality indicators; Figure S3: Spearman's correlation between seed quality indicators. TSW, thousand-seed weight; GR, germination rate; GP, germination potential; *** indicated a extremely significant correlation between indicators ($p < 0.001$); Figure S4: Monte Carlo permutation test between seed and sprout groups for genetic diversity parameters; Figure S5: Monte Carlo permutation test between seed and sprout groups in variance components from AMOVA; Table S1: The coordinates of seven populations; Table S2: Primer sequences, expected fragment size, and annealing temperature of twelve SSR markers; Table S3: The mating system parameters within families.

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