Landscape characteristics determine the gene exchange pattern of Opisthopappus species in the Taihang Mountains

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Abstract

Landscape features are effective geographical barriers resulting inpopulation differentiation of plant species. Taihang Mountains in China possess complexly geographical topology and specific landscape characteristics. Two closely related syntopic Opisthopappus species, mainly distribute in different areas of Taihang Mountains, respectively. How the landscape of Taihang Mountains affects the gene exchange between these two species still unclear. Combined SNP data from restriction-site associated DNA sequencing (RAD-seq) and recently developed landscape genetic methods (EEMS, Samβada, LFMM), we conducted a landscape genetic analysis of these two species. It found that the diversity of O. longilobus was higher than that of O. taihangensis, the gene flow was mostly from north to south along Taihang Mountains. However, a general north–south gene exchange barrier between O. longilobus and O. taihangensis was detected. Among the landscape factors of Taihang Mountains, eight was found to be the important ones, including average precipitation in August, October, and November, solar radiation in August, soil PH, built-up land, rain-fed cultivated land, and workability. And these eight factors were closely related to the occurred barriers, indicating that climatic conditions and human activities rather than geographical environment resulted in these barriers. Twenty-nine selected SNPs were identified to be significant correlated with the eight factors, especially average precipitation in November. Thus, the average precipitation in November could be regarded as an ecological indicator for O. longilobus and O. taihangensis. The results revealed the effect of landscape features on two species and the adaption on the landscape environment of Taihang Mountains during the long-term evolution.

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ABSTRACT

Natural and man-made landscape features are effective geographical barriers resulting inpopulation differentiation of plant species. Taihang Mountains in China possess complexly geographical topology and specific landscape characteristics. Opisopappus taihangensis and O. longilobus, two closely related syntopic Opisthopappusspecies, mainly distribute in the south and north of Taihang Mountains, respectively. How the landscape of Taihang Mountains affects the migration / gene exchange between these two species still unclear. Combined SNP data from restriction-site associated DNA sequencing (RAD-seq) and recently developed landscape genetic methods (EEMS, Samβada, LFMM), we conducted a landscape genetic analysis of these two species. It found that the diversity of O. longilobus was higher than that of O. taihangensis. the gene flow was mostly from north to south along Taihang Mountains. However, a general north-south gene exchange barrier between O. longilobus and O. taihangensiswas detected. Among the landscape factors of Taihang Mountains, eight was found to be the important ones, including average precipitation in August, October, and November, solar radiation in August, soil PH, built-up land, rain-fed cultivated land, and workability. And these eight factors were closely related to the occurred barriers, indicating that climatic conditions and human activities rather than geographical environment resulted in these barriers. Twentynine selected SNPs were identified to be significant correlated with the eight factors, especially average precipitation in November. Thus, the average precipitation in November could be regarded as an ecological indicator for O. longilobus and O. taihangensis. The results revealed the effect of landscape features on two species and the adaption on the landscape environment of Taihang Mountains during the long-term evolution.

Keywords:

Opisthopappus

Landscape

 SNP

Gene exchange

Introduction

Landscape ecology is an interdisciplinary scientific discipline that focuses on spatial pattern and heterogeneity, and specifically their characterization and description over time, their causes and consequences (Francis et al., 2022; Wu, 2013). By using ecosystem principles and system methods, landscape ecology mainly focuses on landscape structure and function, landscape dynamic change and interaction mechanism, landscape spatial pattern, structure optimization, rational utilization and protection, and human management (Jiang and Li, 2021; Li et al., 2021a). In recent years, more researches have been conducted on land use, human experience and ecosystem services (Hersperger et al., 2021; Lester et al., 2021; Song et al., 2017). Nevertheless, with the constant climate changes and increasing human activities, the landscape configuration of different regions is continuously changing (Aylward et al., 2020; Wang et al., 2021c). This change could affect the ecological and evolutionary processes of organisms, and even ecosystems. During these processes, the organisms begin to respond the habitat change caused by the landscape changes and gradually adapt to and evolve in the changed landscapes (Fisher et al., 2021; Wang et al., 2021b).

Landscape changes can result in the appearance or disappearance of dispersal corridors for species or populations. Landscape factors would affect population connectivity by correlating gene exchange. The patterns of dispersal and gene exchange are key components shaping population or species genetic structure and variation model over small and large spatiotemporal scales (Polato et al., 2017). However, less is known about the ecological role of migration or gene exchange. Landscape-genetic approaches can infer landscape effects on dispersal and migration by examining relationships between genetic differentiation and landscape conditions among sampling locations (Anderson et al., 2010; Savary et al., 2021).

Landscape genetics has been a constantly growing field that combines population genetics, landscape ecology, and spatial analytical techniques to quantify the effects of landscape on micro-evolutionary processes. Recent studies have shown that about 80 % researches in landscape genetics were focused on animals. In comparison, there were only about 14.5 % reaearches were on plants, possibly because the mechanism of plant pollen and seed propagation makes the study of gene exchange at plant landscape-scale more complex than animals (Song et al., 2017). However, the plant landscape genetic research is essential for biodiversity because it can provide a powerful tool for protecting important ecosystems, managing vegetation protection units, and charactering the local adaptation and the evolution of species (De Kort et al., 2021).

Taihang Mountains (N34°34'-40°43', E110°14'-114°33') is a vital mountain range and geographical boundary in northern China, one of the second and third stepped boundaries of China's terrain, which stretches about 400km from northeast to southwest (Shoubao et al., 2019; Wu, 2020). The terrains of the Taihang Mountains are higher in the north and lower in the south, and composed of a variety of rocks, cliffs, and many rivers, which formed various and unique landscapes in the Taihang Mountains. In Taihang Mountains, there are more than 1,290 wild species, of which 34 species were listed as a national priority protection species, and 23 species were rare and endangered plants. Thus, this mountain has already been regarded as one ideal research site for many ecological researchers (Wang, 2020). Nevertheless, most of the researches currently paid attention to the geology, geomorphology, and culture on the Taihang Mountains. There are few reports about the impact of the landscape features on the plants in this area.

Opisthopappus genus only lives on the Taihang Mountains cliffs, presenting excellent ecological, medicinal, and economic values. Unfortunately, the individual number of this genus is decreasing mainly due to artificial harvesting and has been listed as China's national II-protected plants. It contains two species: *Opisopappu-staihangensis* and *O. longilobus*. *O. taihangensis*mainly distributes in the south of the Taihang Mountains, covering Henan and Shanxi provinces. *O. longilobus* mainly grows at northern of the Taihang Mountains, including the border of Hebei province and Shanxi Province (Wang et al., 2022).

Our wild investigation found that the shortest distance between *O. taihangensis* and *O. longilobus* is only about 25 km in the central areas of Taihang Mountains. However, the two species showed not only morphologically but also genetically differences. The morphological difference is mainly manifested in the leaves, whereas the morphologies of flowers show little difference. According to the previous studies, the populations of the two species could be clearly separated into two groups with distinct genetic differentiation, which might be the consequence of *Opisthopappus* 's long-term adaptation to the environment of Taihang Mountains(Lin et al., 2013). However, how do the unique landscape features of Taihang Mountains affect the dispersal/ migration and differentiation of the two species? In addition, the geographical and geomorphic topological structure caused by mountain uplift often provides pathways for connectivity, gene exchange, and expansion or contraction of species' populations in its distribution ranges (Hou et al., 2020). Previous studies found that there was a limited gene exchange/flow between *O. taihangensis* and *O. longilobus* (Ye et al., 2021). What landscape factors would prevent gene exchange/flow between these two species, abiotic environmental factors, biotic environmental factors, or conbination of these factors? Is the gene exchange pattern between *Opisthopappus* species a interplay of landscape changes and species? These questions are still unanswered.

In this study, we combined the reduced representation genome data and landscape characteristics of Taihang Mountains to analyze the genetic characteristics of *O. taihangensis* and *O. longilobus*, to reveal the potential gene exchange pattern among these two species, and to analyze the effects of landscape factors and then explore the possible interplay of landscape - species. The results would guide scientific protection and utilization of *Opisthopappus* species and play a theoretical clue for studying other species in the Taihang Mountains.

Materials and Methods

2.1 Materials collection

The fieldwork had been approved by the Chinese government and implemented following the People's Republic of China laws. Each participant had a letter of introduction from the College of Life Sciences, Shanxi Normal University. The samples in this study were taken along the Taihang Mountains from August to October and mainly covered the distribution ranges of *Opisthopappus* species. The elevation, longitude, and latitude information of each site were recorded by GPS and shown in Table 1(Papadopulos et al., 2014).

Table 1

The coordinates of sampled populations of Opisopappus taihangensis and O. longilobus.

Population	Group	Lon	Lat
XTDXG	OLa	113.9412	37.2113
BWD	OLa	114.089	37.066
JNH	OLa	113.9814	36.992
QTH	OLa	113.9251	36.8625
SLD	OLa	113.9253	36.8622
XTSK	OLb	114.2216	36.591
LFS	OLb	113.7612	36.499
HLT	OLb	113.598	35.9635
THDXG	OLb	113.585	35.8515
GLC	OTc	113.6117	35.736
WML	OTc	113.585	35.6914
LSZT	OTc	114.016	35.6219
DSC	OTc	113.4335	35.56917
GS	OTd	113.5642	35.5669
BQ	OTd	113.4916	35.479
JYS	OTd	113.34	35.392
FXD	OTd	112.8853	35.2776

OL: Opisopappus longilobus ; OT: Opisopappus taihangensis .

The individuals growing in the same area were considered as a "population", and 160 samples from 17 populations were collected (Table 1). Five to ten individuals were randomly selected from each population, with at least 5m between individuals. Fresh, mature, and healthy leaves were sampled and stored in sealed bags containing silica gel, then sent back to the laboratory.

According to the geographical distribution of two species, the sampled populations were divided into four groups: the northern group of *O. longilobus* (OLa: including XTDXG, BWD, JNH, QTH, SLD populations), the southern group of *O. longilobus* (OLb: containing XTSK, LFS, THDXG, HLT populations), the northern group of *O. taihangensis* (OTc: including GLC, WML, LSZT, DSC populations) and the southern group of *O. taihangensis* (OTd: containing GS, BQ, JYS, FXD populations) (Fig. 1).



Fig. 1. Sampled sites of Opisopappus taihangensis and Opisopappus longilobus .

The sampled sites of OLa, OLb, OTc and OTd were distinguished by pink, blue, yellow, and red respectively and visualized on the map.

2.2 SNPs screened and acquisition

Genomic DNA from 160 individuals leaves of *Opisthopappus* was extracted by the modified CTAB method (Papadopulos et al., 2014). The quality of DNA was evaluated by 0.8% agarose gel electrophoresis and an ultraviolet spectrophotometer.

In order to construct genome sequencing library to detect single nucleotide polymorphism (SNPs), the extracted genome DNA was fully enzymatically cut by using EcoRI+BfaI restriction endoenzyme. Too large and too small fragments after enzyme digestion were removed using VAHTSTM DNA Clean Beads, and then were ligased with the P1 connector and P2 connector. PCR reactions were performed to enrich the sequencing library template. PCR products were purified by VAHTSTM DNA Clean Beads and then sequenced on Illumina HiSeq sequencer using 2×150 bp double-terminal sequencing by Shanghai Personalbio Technology CO., LTD.. The quality of data was controlled using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) (Schmidt et al., 2021). Reads with Q20 [?] 90% and Q30 [?] 85% were screened from raw data to obtain high quality data. Trimmed reads were mapped to the *Chrysanthemum* genome version 2.0 (http://www.amwayabrc.com/index.html) using BWA and SAMtools program "rmdup" to remove duplications. Only reads with at least 85 % match to the reference genome were retained(Contaldi et al., 2021).

After sequencing, SNPs were detected by GATK v3.8 software (do Valle et al., 2016; Peter et al., 2018). All SNPs were screened from 160 individuals and saved as VCF files. Subsequently, the SNPs with deletion rates over 5% were removed by Plink 1.9 with Geno and MAF commands, and that with 1% minor interference frequency was selected. Finally, the SNPs with P<0.000001 were screened out with the hardy - Weinberg balance test performed by hardy command (Purcell et al., 2007).

2.3 Genetic characteristics estimated

The observed heterozygosity $(H_{\rm O})$ and average heterozygosity $(H_{\rm S})$ were calculated using STACKS of different populations, groups, and species based on the screened SNP data (Rochette et al., 2019). $F_{\rm IS}$ value (inbreeding coefficient) anong populations, groups, and species was evaluated using the "boot. ppfis" function of R package hierfstat with 999 bootstrap values (Goudet et al., 2015). The mean allelic richness (i.e., mean number of alleles per locus, Ar) was calculated with the function "allel.rich" of R package PopGenReport (Adamack and Gruber, 2014). The proportion of shared alleles between populations, groups and species was estimated with the "pairwise.propShared" function of the R package PopGenReport (Song et al., 2017). The IDW model of GIS was used to visualized the results on the map (Yu et al., 2014).

The genetic variations ($F_{\rm ST}$) among populations, among groups, and between species were estimated by Arlequin v3.5 with default parameter (Excoffier and Lischer, 2010). $F_{\rm ST}$ values of 0- 0.05, 0.05-0.15 and 0.15-0.25 indicated a little genetic differentiation, moderate genetic differentiation, and extensive genetic differentiation among populations, respectively; $F_{\rm ST} > 0.25$ indicated a substantial genetic differentiation among populations.

2.4 Gene exchange/flows and migration

Bayesian method implemented in the STRUCTURE v.2.3.4 was used to estimate the likely number of genetic clusters (K) for *Opisthopappus* species (Papadopulos et al., 2014; Pritchard et al., 2000). A range of K values was planned from K = 1 to 10. Each number of K clusters was assessed in 5 independent runs, with a burn-in of 100,000 MCMC steps, followed by an additional 100,000 MCMC steps (Kunde et al., 2020; Morris-Pocock et al., 2016). Then the resulted files were uploaded to the "STRUCTURE HARVESTER" website (http://taylorO.biology.ucla.edu/struct_harvest/) (Earl and VonHoldt, 2012). And the structure cluster diagram was drawn by the "clumpak" website (http://clumpak.tau.ac.il/) (Furlan et al., 2013; Kopelman et al., 2015).

To analyze the gene exchange among populations, groups and species, the effective migration rate was used in this study. The effective migration rate is the migration rate (M) and was calculated using BayesAss 3.0 software (Wilson and Rannala, 2003). When running, the parameters were set at 0.50 allele frequency, 0.50 inbred line number, 0.50 migration rate, 100 seed, program 10000000 times, 1000000 number of iterations, 1000 sampling interval to ensure the reliability and accuracy of M value (Morris-Pocock et al., 2016).

EEMS (Estimate effective Migration Surface) uses the concept of effective migration to model the relationship between genetics and geography, and it can output an "estimated effective migration surface" (Petkova et al., 2016), namely, a visual representation of population structure that can highlight potential regions of higher-than-average and lower-than-average gene flow. Thus, the migration patterns of *O. taihangensis* and *O. longilobus*, from the group, population to individual, in the Taihang Mountains were analyzed through the effective migration surface by EEMS software (Mahtani-Williams et al., 2020).

Firstly, VCFtools software converted SNPs data into bed, fam, and bim files (Danecek et al., 2011). The "BED2DIFFs" program provided by the EEMS model on the Ubuntu platform converted the genetic differentiation matrix file from SNPs into diffs. Meanwhile, each population's latitude and longitude coordinates were arranged in the same order as SNPs data and made a coord file.

In addition, the habitat polygon per dataset (coord file) was obtained using Google Maps API v3 Tool (*http://www.birdtheme.org/useful/v3tool.html*) and plotted using the R package rEEMSplots (Petkova et al., 2016). A distribution map of genetic characteristics of *Opisthopappus*species was also outputted by EEMS based on the above data (Mahtani-Williams et al., 2020).

2.5 Landscape factors extraction

Landscape factors required for data mainly came from the FAO (http://www.fao.org/home/zh/) and Worldclim (https://worldclim.org/) website (Mahtani-Williams et al., 2020). For the Taihang Mountains, the climate data was (1970-2000) mainly from WorldClim 2.1 (www.worldclim.org) and land data was from the United Nations Food and Agriculture Organization (www.fao.org). Total 107 landscape factors were obtained, such as monthly mean temperature (), maximum temperature (), rainfall (mm), wind speed (m/s), vapor pressure (kpa), solar radiation (kJ /m2*d), and land development and utilization (Support files 1).

For each sampled location, the climatic landscape factors were extracted from the downloaded raster dataset and ASCII dataset using the "rgdal" and "raster" packages in the R software (Bivand et al., 2015; Hijmans et al., 2015) (Support files 1).

Because the multicollinearity of factors can lead to the faulty of modeling, the highly correlated environmental predictors were removed. And the variance inflation factor (VIF) was calculated by R package "usdm" for each landscape factor to detect the current linearity, and then the factors with VIF < 5 were kept (Mahtani-Williams et al., 2020; Papadopulos et al., 2014; Ruan et al., 2021) (Support files 2).

The principal component analysis was performed with Origin 2022 software (https://www.originlab.com/) to determine important landscape factors that contributed to the *O. longilobus* and *O. taihangensis*. Subsequently, the screened factors were also mapped by Origin 2022 software (Fig. 2).



Fig. 2. PCA of landscape factors of Taihang Mountains.

PCA analysis was performed on 160 individual landscape factors with VIF<5.

Finally, eight landscape factors were retained, namely average precipitation in August, average precipitation in October, average precipitation in November, built-up land (residential and infrastructure), rain-fed cultivated land, workability (restricted site management), solar radiation in August, and soil PH.

$2.6 \ F_{ST}$ outliers filtered and selected SNPs identified

 $F_{\rm ST}$ outliers generally show the selected genes or loci among populations. To identify the selected SNPs in the two *Opisthopappus* species, BAYESCAN 2.1 software was used to filter the $F_{\rm ST}$ outliers (Fischer et al., 2011; Foll et al., 2010; Ruan et al., 2021). Prior odds of the selection model were set at 10,000 to reduce false-positive results under a variety of demographic events. A logarithmic scale for model choice of selection over neutrality was defined as: substantial (log10PO > 0.5, 0-0.05), strong (log10PO > 1.0, 0.05-0.15), very strong (log10PO > 1.5, 0.15-0.25) and decisive (log10PO > 2, $F_{\rm ST}$ >0.25). A gene or locus with log10PO > 0.5 was considered as a potential selective outlier under natural selection (Feng et al., 2015). Finally, 29 genes/loci identified based on the BayeScan were considered as putative SNPs under selection. These SNPs were retained for the subsequent landscape features association analysis. Then the filtered SNPs were extracted from the VCF file.

2.7 Association of SNPs with landscape factors

The SNP associations with landscape factors were assessed using Samβada v.0.9.0 and latent factor mixed model (LFMM) software (Chien et al., 2020; Feng et al., 2015; Ruan et al., 2021). Samβada builds logistic regressions to estimate an individual's probability of presenting a particular molecular marker depending on the landscape factors that characterize its sampling site (Li et al., 2019; Vargas-Mendoza et al., 2016).

In order to accurately describe the landscape factors of each population, the eigenvalues of the first four principal components of principal component analysis (PC1-4) were chosen, which explained 77.04% of the total landscape features. In Samßada, the effect of each landscape factor was tested by adding one factor at a time to the population landscape factors (dimensionP+1), and the more likely model was assessed (without or with the landscape factors). For each test model, Samßada created an output file containing the model parameters, logarithmic likelihood, G score, Wald score, AIC, and BIC. To ensure the model's accuracy, all the models were screened according to the AIC value (Mahtani-Williams et al., 2020; Stucki et al., 2017). Then the first 29 valid models were selected with the smallest AIC value, and the proportion of each factor in these 29 models was counted. And the 29 models involved a total of three genes among selected SNPs. These genes were subsequently subjected to carry KEGG annotation (https://www.genome.jp/kegg/).

LFMM is a hierarchical Bayesian hybrid model, which considers the background of population structure as the random effect of population history and isolation by distance model, and through the potential K value of population structure (Frichot et al., 2013; Wang et al., 2017). In LFMM, the genetic data matrix was tested based on a z-score as a fixed effect. The number of possible factor K was set to 2 (according to the Structure results). LFMM ran 5 times with 10,000 iterations in the Gibbs sampling algorithm and a burn-in period of 5,000 cycles for each K value. Z-scores from five independent replicate runs were combined using Fisher–Stouffer method, and the P values were adjusted using the genomic inflation factor (λ). P values were further adjusted based on an FDR correction of 1 % using the R 'qvalue' package to get Q values (Li et al., 2019).

3. Result

3.1 Genetic characteristics about two species

For the all 17 populations, the HLT population had the largest genetic diversity values, A_r , H o and H s were 1.262, 0.138, 0.104 respectively (Table 2). SLD population had the minimum values, A_r and H_s were 1.198 and 0.082 respectively. However, WML population showed a lowest H_o value of 0.116.

Table 2

The diversity index of different populations, groups and species.

	Ar	$F_{\rm IS}$	Η _O	$H_{\rm S}$	Group
O. longilobus	1.677925211	-0.009398987	0.128320265	0.103881423	
O. taihangensis	1.60747889	-0.025400838	0.123118215	0.095717081	
OLa	1.455729795	-0.102206649	0.125995175	0.095882702	O. longilobus
OLb	1.528106152	-0.046989581	0.130645356	0.106598215	O. longilobus
OTc	1.447285887	-0.109511515	0.121399276	0.089817419	O. taihangensis
OTd	1.476718938	-0.064340714	0.124837153	0.098722002	O. taihangensis
XTDXG	1.226516637	-0.277125605	0.123884198	0.08855696	OLa
BWD	1.250165271	-0.256766561	0.132086852	0.093288251	OLa
JNH	1.23777694	-0.251719338	0.123546441	0.088993848	OLa
QTH	1.227503016	-0.350401816	0.128636912	0.089939686	OLa
SLD	1.197828709	-0.399797436	0.120289505	0.081712907	OLa
XTSK	1.200741214	-0.441942111	0.131338963	0.085991653	OLb
LFS	1.238123785	-0.240439894	0.125500603	0.091344391	OLb

	Ar	$F_{\rm IS}$	H _O	$H_{\rm S}$	Group
HLT	1.261897622	-0.232742502	0.137587455	0.103819638	OLb
THDXG	1.258555485	-0.21265296	0.128154403	0.095575103	OLb
GLC	1.230312214	-0.261028304	0.123039807	0.087653462	OTc
WML	1.229970829	-0.212619449	0.1160193	0.084477467	OTc
LSZT	1.222244851	-0.304460566	0.124969843	0.087079952	OTc
DSC	1.216224376	-0.318906335	0.121568154	0.084209264	OTc
GS	1.219185983	-0.352063424	0.128371532	0.086546297	OTd
BQ	1.202721287	-0.30224822	0.117659831	0.083091242	OTd
JYS	1.229036986	-0.259342005	0.122798552	0.087766321	OTd
FXD	1.234433893	-0.263066884	0.130518697	0.096115537	OTd

Ar: allelic richness; $H_{\rm O}$: observed heterozygosity; $H_{\rm S}$: average heterozygosity; $F_{\rm IS}$: inbreeding coefficient.

Among the four group of OLa, OLb, OTc and OTd, A_r showed OLb (1.528) > OTd (1.477) > OLa (1.456) > OTc (1.447), H_s was OLb (0.107) > OTd (0.099) > OLa (0.096) > OTc (0.090), and H o showed OLb (0.131) > OLa (0.126) > OTd (0.125) > OTc (0.121). The OLb group had highest genetic diversity among the four groups.

Between the two species, the values of A_r , H_o and H_s of *O. longilobus* were 1.678, 0.128 and 0.104 respectively, which were higher than 1.608, 0.123 and 0.096 of *O. taihangensis*. More, all the diversity index of *Opisthopappus* species showed that a trend of high in the middle and northern and southern end areas and low in other regions (Fig. 3).



Fig. 3. The distribution of genetic characteristics of *Opisopappus taihangensis* and *Opisopappus longilobus* .

a: The distribution of Ar values; b: The distribution of F $_{\rm IS}$ values; c: The distribution of H $_{\rm O}$ values; d: The distribution of H $_{\rm S}$ values.

The $F_{\rm IS}$ in this study were all negative (Table 2), with a mean $F_{\rm IS}$ of -0.290 for all 17 populations, -0.081 among four groups, and -0.0174 between two species. Among these 17 populations, WML (-0.213) population had a largest $F_{\rm IS}$ value, while XTSK (-0.442) had a smallest $F_{\rm IS}$ value.

From the population level, obvious genetic differentiation occurred among populations ($F_{\rm ST} > 0.15$) (Table S1). At the group level, there was a significant differentiation among four groups. The $F_{\rm ST}$ value was 0.31731 between OLa and OTc, 0.31418 between OLa and OTd, 0.30038 between OLb and OTc, and 0.29795 between OLb and OTd. For *O. taihangensis* and *O. longilobus* species, the $F_{\rm ST}$ was 0.29418, which also showed a significant differentiation between two species.

3.2 Gene exchange /flow and migration pattern

Gene exchange results showed that the migration rates from the north groups to the south groups (0.0156-0.2958) were generally higher than that from the south groups to the north groups (0.0076-0.0151). Between OLb and OTc groups, the migration rate was lower from north to south (0.0073) than from south to north (0.0227). Within the group level, the gene migration rates within population of OLa group (0.5808) were much lower than that of the remaining three groups (above 0.95) (Fig. 4).



Fig. 4. Migration rate among four group, between species.

According to the method of EVANNO, a maximum likelihood ([?]K) was used to determine the value of K. In this study, when K = 2, [?]K reached the maximum value (Fig. 5). The four groups were divided into two species. Unexpected, the potential K value for the OLb group was not 2 but 4. Mixed individuals were found between and within the four groups, indicating that there have gene flow among groups and species.



Fig. 5. Structure analysis of Opisopappus taihangensis and Opisopappus longilobus .

a: Total 17 populations divided into OLa, OLb, OTc and OTd groups; b: The populations of OLa group; c: The populations of OLb group; d: The populations of OTc group; e: The populations of OTd group.

The EEMS results (Fig. 6) revealed that a significant migration barrier occurred among populations at the junction of two species along the Taihang Mountains. Interestingly, the migration channel for the individuals of the two species was not along with the mountainous areas but more inclined to the plains in the Taihang Mountains. Additionly, the populations closest to the migration barrier had much higher migration rate than the average of whole species. The areas with the highest genetic diversity tended to be in the areas with low migration rates. Neverthless, the areas with the highest genetic diversity of O. longilobus was just in the obstacle areas between O. taihangensis and O. longilobus.



Fig. 6. Effective migration patterns of Opisopappus taihangensis and Opisopappus longilobus.

a: Posterior mean migration rates m (on the log10 scale); b: Posterior mean diversity rates q (on the log10 scale)

3.3 The relationship between SNPs and landscape factors

A total of 29 SNPs were identified under selection based on the $F_{\rm ST}$ outlier approach implemented in BayeScan software (q-value < 0.01) (Support files 3).

Samßada results showed that, in the first 29 (AIC < 17) effective models, seven landscape factors of Taihang Mountains, average precipitation in August, average precipitation in November, built-up land (residential and infrastructure), rain-fed cultivated land, workability (restricted site management), solar radiation in August, and soil PH, were closely related with the selected SNPs, which accounted for 13.8% of total variation revealed by BAYESCAN. Average precipitation in October in Taihang Mountains accounted for 3.4%, was significantly correlated with 29 SNP loci (Support files 5).

LFMM analysis revealed that 27 of 29 SNP loci were associated with average precipitation in August (8.66%), average precipitation in November (15.75%), built-up land (residential and infrastructure) (11.81%), rainfed cultivated land (11.81%), workability (restricted site management) (11.81%), solar radiation in August (12.6%), soil PH (14.17%), average precipitation in October (13.39%) (Table 3).

In total, 29 selected SNPs were strongly associated with landscape features of Taihang Mountains revealed by Samβada and LFMM analysis, especially average precipitation in November.

The identified selected SNPs were mainly enriched in Carbohydrate metabolism, Energy metabolism, Translation, Signal transduction and Transport and catabolism. The genes related with these SNPs regulate Glycolysis / Gluconeogenesis, Pentose and glucuronate interconversions, Ribosome, MAPK signaling pathway. In particular, MAPK signaling and plant hormone signal transduction pathway were found to be involved in the three genes based on samβada analysis.

4. Discussion

4.1 Landscape features affect genetic characteristics of Opisthopappus species

The genetic diversity of O. longilobus was higher than that of O. taihangensis , which is consistent with previous studies (Ye et al., 2021). Being an ancestral species of *Opisthopappus* , more genetic variation might be acculmated into this species than its descendant O. taihangensis . Furthmore, the OLb group of O. longilobus had the highest genetic diversity than other three groups. It suggested that this group might be a diversity centre for O. longilobus, even for the whole *Opisthopappus* genus, especially the areas of HLT and THDXG populations, which had the highest genetic diversity among all populations (Ren et al., 2022) (Table 1).

For the two groups of *O. longilobus*, the OLa group had a relative lower genetic diversity than OLb group (Table 1). The populations of OLa group are at the margins of geographic range. Generally, the marginal populations are often thought to be poorly adapted to their environment (Bontrager and Angert, 2019). Howover, a gene exchange/flow can provide beneficial genetic variation and may facilitate adaptation to environmental change (Li et al., 2021b; Wood et al., 2021). This is because that gene flow is expected to increase heterozygosity and reintroduce variation that can allow for masking or purging of fixed deleterious alleles (Ferrer et al., 2021; Muola et al., 2021). Unexpected, the OTc group (central populations) of *O. taihangensis* presented a slightly lower genetic diversity than that of OTd group (marginal populations) (Table 1). This might be related with the relative higher gene flow from OTd to OTc (Fig. 3).

Multilocus analysis resulted in significantly negative $F_{\rm IS}$ values for the inbreeding coefficient, indicating the presence of heterozygote excess for *Opisthopappus* species. The phenomenon of excess heterozygotes (e.g., *Carapa procera*, *Dioon edule*, *Prunusavium*) has widely been observed in other species. In general, the main reasons for the excess of heterozygotes are low number of individuals in the breeding population, overdominance, stepwise selection for homozygotes and negative assortative mating (Million, 2021; Stoeckel et al., 2006). The excess of heterozygotes implied that the species possessed a rich genetic diversity that favored the adaptation of the species to different environments (Tay Fernandez et al., 2021; Theodoridis et al., 2021). Because of the lack

Table 3

The correlation between 29 selected SNP and landscape factors.

	average precipitation in august	average precipitation in october	average precipitation in november	b
gen1	0.3564594	0.4753122	0.006177823	0
gen2	0.2183109	0.000141346	0	0
gen3	0.1303628	0.000245717	0	0
gen4	0	1.70063E-06	0.01942746	1
gen5	0	0	0.002252055	0
gen6	0.06396126	0.001787113	0	0
gen7	0.4069805	0.000705721	0.004220458	0
gen8	0.2337786	0.2474782	0.09337141	1
gen9	0	0.01430407	0.09894669	0
gen10	0	0.8239689	0.001610374	0
gen11	0	1	0	0
gen12	0.002481249	0.9143786	0.001573389	0
gen13	0.7255149	0.01910583	0	0
gen14	0.01420107	0.001291673	0.1071167	0
gen15	0.001561764	0.02566867	0	0
gen16	0.3390629	1	0.5439095	0
gen17	1	0	0	0
gen18	1	1	0.07689282	0
gen19	3.78E-06	5.76463 E-05	2.37507E-12	0
gen20	5.37 E-05	0.001238759	1.6393E-07	0
gen21	0.8075378	0	0.01226667	0
gen22	0.4053446	0.2529489	0	0
gen23	0.07772289	0.001520768	0	0
gen24	0	1.93849E-10	0.4535689	0
gen 25	0.2041402	1	0.114697	0
gen26	0.2057847	0.9968121	0.114459	0
gen27	0.2615698	0.8772384	0	0
gen28	0.2394105	0.7774969	0.1029429	0
gen29	1	0	0	0
totel Q < 0.01	11	17	20	1
Proportion	8.66%	13.39%	15.75%	1

of information on mating styles and reproductive fitness of the Opisthopappus, we cannot determine the likely causes for the heterozygote excess and further research is needed.

4.2 Landscape factors determine gene exhchange pattern of Opisthopappus species

Maintaining an adequate migration rate is usually beneficial because the populations of species experiencing limited gene flow would be susceptible to loss of genetic diversity or genetic variation due to inbreeding and drift (Bansept et al., 2021; Kottler et al., 2021). Lower genetic diversity or genetic variation may make species difficult to adapt to changing environmental conditions, leading to local extinctions (Matzrafi et al., 2021; Wellband et al., 2021).

The gene exchange degree within *Opisthopappus* species present a pattern mainly from north to south. The higher altitude of the northern in Taihang mountains may contribute to some extent to the genes spread of the *Opisthopappus* species. Meanwhile, the pollens and seeds of *Opisthopappus* are small and light, and easily spread with wind. Each year during the breeding period of the *Opisthopappus*, the wind by Siberia weather in the Taihang Mountains could be an important factor that facilitate the gene transmission from north to south (Wadl et al., 2022).

Although there is gene exchange between two species and four groups, the gene exchange was extremely limited (Fig. 4, 5 and Table S1). This indicated the existence of a gene exchange barrier between *O. longilobus* and *O. taihangensis* or among groups (Fig. 6). Whilist, no obvious topographic obstacles were observed at the barrier between *Opisthopappus* species. Thus, it suggested that the factors hindering gene migration among populations might not be simple topographic landscape factors but other factors of Taihang Mountians landscape (Lu et al., 2021; Zhang et al., 2021). Taihang Mountains have many deep gullies and diverse landforms, which could result in diverse micro-landscape in different regions. This landscape heterogeneity could produce heterogeneous habitats for different populations of species and bring the spatial isolation for gene exchange.On the other hand, gene exchange/flow is often considered a source of maladaptation because it can limit genetic and phenotypic differentiation and reduce mean fitness in a population (Youngblut et al., 2015). Limited gene exchange/flow occurred in *O. longilobus* and *O. taihangensis* might be benifical, even when populations are geographically disparate, because gene flow can allow for the spread of environment-specific beneficial alleles that arise in a single population that could bring the differentiation and adaption (Ellstrand, 2014; Morente-López et al., 2021).

Meanwhile, the gene exchange barrier was located at the juncture of the Hebei, Shanxi, and Henan provinces. The areas of this barrier were suspected to be closely related to human activities. As the flowers of *Opisthopappus* are beautiful and have good medicinal value, human picking and planting activities may significantly influence migration rates (Blanco et al., 2021; Crispo et al., 2011; Liao et al., 2021).

Among the landscape factors of Taihang Mountains, eight factors were the key ones, mainly including six climatic factors and two human activity factors. These factors might be heterogeneous in different regions and form a climatic isolation and/or a corridor by human activities for the populations of species, and which indicating that these factors are important for the development and growth of *O. longilobus* and *O. taihangensis* (Ma et al., 2018; Shih et al., 2018).

For the two species, their flowering begin from June to September. And their seeds develop during September and November. During the development of breeding organs, they need accumulated temperature and precipitation, thus four climatic factors, average precipitation in August, average precipitation in October, average precipitation in November, and solar radiation in August, would play an important role. Except for the above factors, built-up land and rain-fed cultivated land factors also significantly impacted the *Opisthopappus* (Table 3). These two human activities are destructive to the environment that has a long-term occupation and large-scale deep-level transformation on the natural landscape especially physical and chemical properties of the land, which would result in the changes of land workability and soil PH.

Based on the results of Samßada and LFMM, average precipitation in November might have the greatest influence on the *Opisthopappus* (Shih et al., 2018). In the reciprocal transplant, it was found that too much or too little precipitation would lead to seed growth with cavitation. Therefore, it is speculated that the precipitation in November may be regarded as a ecological indicator for the *Opisthopappus* two species (An et al., 2020; Chen et al., 2021; Wang et al., 2010).

Meanwhile, among the pathways mainly enriched by the identified selected SNPs, MAPK signaling and plant hormone signal transduction pathways were significant ones. The two pathways are directly or indirectly related to resistance of plant. In the growth and development of *Arabidopsis thaliana*, it has been shown that MAPK signaling pathway was related to environmental response (Fengbo, 2019). Plant hormone signal transduction pathway has been shown to be related to the plant height through affecting the cell elongation (Haruta and Sussman, 2017). Plant height can affect the ability of plants to receive sunlight, which might be one reason why solar radiation in August is an important factor for *Opisthopappus* species (Wang et al., 2021a). Thus, it could be hypothesized that precipitation, solar light and soil PH might paly a signal role that activate or inhibit the relative gene express, thereby, bring the adaption of two species to the different landscapt in the Taihang Mountains.

Conclusion

Landscape heterogeneity often plays a vital role in the ability of organisms to disperse or not to disperse. Habitat fragmentation, both natural and artificial, adversely affects the connectivity and persistence of populations. Various landscape features of the Taihang Mountains could make the different environmental conditions and fragmentized habitat of each population or species of *Opisthopappus*. With the changes of climate, and human activities, landscape heterogeneity lead to the barrier among *Opisthopappus* species and hinder the gene exchange among the population and promote the differentiation and adaptation among populations ever between species.

CRediT authorship contribution statement

Hao Zhang: Methodology, Writing – review & editing. Hang Ye: Methodology, Editing. Li Liu: Methodology. En Zang: Analysis, Investigation. Qiyang Qie: Sorting data, Investigation. Shan He: Investigation. Weili Hao: Analysis, Investigation. Yafei Lan: Investigation. Zhixia Liu: Investigation. Genlou Sun: Supervision, Review & editing. Yiling Wang: Conceptualization, Funding acquisition, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they do not have any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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