Contrasting fine scale genetic structure of two sympatric clonal plants in alpine swampy meadow featured by tussocks

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Tussock is a unique structure in wetland vegetation. Many tussock species reproduce mainly by clonal growth, resulting in genetically identical offsprings distributed in various spatial patterns. These fine scale patterns could affect the mating patterns and thus long-term evolution of wetland plants. Here we contribute the first genetic and clonal structure of two key species in alpine wetlands of the Qinghai–Tibet Plateau: *Kobresia tibetica* and *Blysmus sinocompressus*, using > 5000 SNPs identified by 2b-RAD sequencing. The tussock builder *K. tibetica* has a phalanx growth form but different genets could co-occur within tussock, indicating it's not proper to treat tussock as one genetic individual. Phalanx growth form does not necessarily lead to increased inbreeding in *K. tibetica*. *B. sinocompressus* has a guerilla growth form, with the largest detected clone size of 18.32m, but genets at the local scale tends to be inbreeded offsprings. Our results highlight that the contemporary advantage of *B. sinocompressus* facilitates the combination of clone expansion and fast seedlings, but its evolutionary potential is limited by the input genetic load of original genets. Tussocks of *K. tibetica* are more diverse and valuable genetic legacy of former well developed wet meadow worthy of conservation attention.

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Abstract: Tussock is a unique structure in wetland vegetation. Many tussock species reproduce mainly by clonal growth, resulting in genetically identical offsprings distributed in various spatial patterns. These fine scale patterns could affect the mating patterns and thus long-term evolution of wetland plants. Here we contribute the first genetic and clonal structure of two key species in alpine wetlands of the Qinghai–Tibet Plateau: *Kobresia tibetica* and *Blysmus sinocompressus*, using > 5000 SNPs identified by 2b-RAD sequencing. The tussock builder *K. tibetica* has phalanx growth form but different genets could co-occur within tussock, indicating it's not proper to treat tussock as one genetic individual. Phalanx growth form does not necessarily lead to increased inbreeding in *K. tibetica*. *B. sinocompressus* has guerilla growth form, with the largest detected clone size of 18.32m, but genets at the local scale tends to be inbreeded offsprings. Our results highlight that the contemporary advantage of *B. sinocompressus* facilitates the combination of clone expansion and seedling recruitment, but its evolutionary potential is limited by the input genetic load of original genets. Tussocks of *K. tibetica* are more diverse and valuable genetic legacy of former well developed wet meadow worthy of conservation attention.

Keywords: clonal plant; spatial genetic structure; *Kobresia tibetica*; *Blysmus sinocompressus*; tussock; inbreeding; SNP; Qinghai–Tibet Plateau; clonal structure

1. Introduction

Vegetation is of fundamental importance to alpine ecosystem through processes such as water retention and evapotranspiration [1, 2]. The genetic diversity of plant species is crucial to vegetation as it provides the basis for evolution, especially in face of emerging challenge such as climate change and overgrazing [3]. However, the genetic structure of alpine plants is complicated by the prevalence of clonal growth. Clonal growth is an asexual reproductive strategy that is favored in harsh habitat such as tundra, desert and alpine [4, 5]. During clonal growth, genetically identical offsprings (ramets) are produced vegetatively. Ramets usually remain connected by spacers like rhizomes or solons to form an entire clone (genet). This reproductive mode confers plants the advantages of physiological integration and labor division to persist in extreme environment [6, 7]. Clonal growth also has profound effect on the genetic diversity and evolutionary potential because it affects the mating pattern of plant population. The impacts rely on two main aspects: clone size and spatial arrangement. There have been debates about the effect of size and distribution of clones on genetic structure of clonal plant population (reviewed by Vallejo-Marin *et al*. [8]). In population with clones of uneven size, few and large clones contribute the main part of the genetic component, making the effective population size dramatically smaller than the apparent census population [9]. Additionally, the spatial arrangement of genets differs according to the pattern of clonal growth. Short spacers result in clumped distribution of ramets (phalanx form) while longer spacers can place ramets in various directions over long distances (guerrilla form). The former often produce a separated distribution of genets whereas the latter could present an intermingled pattern. It's been reported that phalanx growth form tends to increase the chances of geitonogamous selfing, particularly with increasing genet size, and consequently increases the risk of inbreeding depression [10-12]. Nevertheless, clonal reproduction is also associated with mass flowering, which increases the opportunity of pollinator visiting. Flowers on the periphery of large clones may receive outcross pollen more easily than smaller clones [13]. Generally, the greatest genetic impact of clonality often occurs at fine spatial scales within populations, due to the limited dispersal capacity of asexual reproduction. It's crucial to find out the the frequency, spatial dynamics and fine-scale genetic impacts of clonal growth before effective conservation management could be issued.

The presence of tussocks is a featured topography in wetland system. In water logged sites, roots of the builder species capture and retain sediments on which plants continue to grow and develop tussocks [14]. Mature tussocks could have expanded basal area and bear many cohabitant species. Facilitation may be the underlying mechanism that promote the coexisting of tussock species. By providing some facilitative effects (e.g. grazing prevention, warmth trap and physical stress relief), tussocks act like fine scale shelters for the sympatric species [15, 16]. Previous studies have shown that individuals in the tussock tend to have more sexual

reproduction events [15, 17]. This phenomenon is of vital importance because the generatively reproducing individuals inside tussocks could serve as a seed source and make critical contribution to the genetic pool of ecosystems preferring clonal growth. However, it is currently unknown if coexisting species in tussocks may have the same genetic structure or mating pattern. Furthermore, individuals from the same tussock are often presumably treated as from the same clone (but see *Carex sempervirens* [18]), which may not be true. The arbitrary clone assignment could result in biased estimation of the mating pattern and gene flow process. Knowledge of the clonality is essential if any inference is to be made about the genetic structure of tussock wetland species.

In wetlands of the Qinghai–Tibet Plateau (QTP), such as swampy meadows, waterlogged areas, and river margins, the vegetation is typically characterized by *Kobresia tibetica* Maxim that is a tussock builder perennial with clumped and erected stems. [19]. *Kobresia* is the key species in the alpine ecosystem of QTP. It's reported that *Kobresia* pastures in the eastern Tibetan highlands occupy 450000 km² and form the world's second largest alpine ecosystem [20]. However, the recent increase in surface soil temperatures and anthropogenic disturbance have led to a deterioration of *K tibetica* swamps and retrogressive successions. A commonly seen successional hygrophyte is *Blysmus sinocompressus* Tang&F.T.Wang. *B. sinocompressus* appears at the early stage of the retrogressive succession and gradually replace *K. tibetica* as the degradation proceeds [20-22]. In contrary to *K. tibetica*, *B. sinocompressus* tends to form drawf and continuous population landscape. These two species both have mixed reproductive strategy. They mainly rely on clonal growth. Limited sexual reproduction occurs when the environment condition is optimal [23, 24]. Previous studies have evaluated the genetic diversity of both species at regional scale [25, 26]. The results have shown that, for both species, the limited sexual reproduction appears competent to maintain the genetic diversity level, and more genetic variation resides in population other than among populations. These results indicate that fine scale genetic structure may exist and play an important role in the gene flow process, which has not been explored yet. Moreover, the specific clonal structure of both species is currently unknown, so the effect of clonality on population genetics and tussock succession remains poorly understood.

Here, we present the first comparative study on the fine scale genetic structure of these two clonal plants, *K. tibetica* and *B. sinocompressus*, which are typical species in the alpine wetland of eastern part of Qinghai–Tibet Plateau. The aim of the study is to find out: (1) the specific clonal structures of these two species; (2) the spatial range on which clonality affects the genetic pattern; (3) the fine scale genetic structure and diversity that could help to explain the successional process of tussock swamp. Specifically, we design a specialized sampling scheme, estimate genotypic diversity, inbreeding level and determine the spatial architecture of clonal

lineage using Single Nucleotide Polymorphism (SNP) loci generated by 2b-RAD sequencing. Spatial autocorrelation analyses were implemented at both the ramet and genet level to assess the impact of clonality on fine-scale genetic structure for each species. We anticipate the findings could be helpful in conservation of the alpine wetland plants and the sustainable management of swampy meadow featured by tussocks.

2. Materials and Methods

2.1. Study area and Species

This study was carried out at the Zoige wetland in the eastern margin of the Qinghai–Tibet Plateau. Altitude in this area ranges from 3400-3600m. The mean annual temperature is about 0.6–1.0 °C. The majority of the precipitation occurs in summer, 580–860 mm annually. Perennial herbaceous species dominate the regional vegetation, of which Cyperaceous species accounts for more than 80% [27]. Our sampling stand was set at a natural wet meadow (33°47′53.59″N，102°57′33.74″E) that is about 25km northward of the Zoige county. This meadow is primarily used as herd pasture with no specific management regime except fencing at boundary. The landscape of the stand is general flat in terrain with scattered distributed tussocks. Total coverage of vegetation is 95% or so by observation.

The vegetation is mainly constituted by two species: *Kobresia tibetica* Maxim. and *Blysmus sinocompressus* Tang & F. T. Wang. Both species are typical endemic hygrophtyes in Qinghai–Tibet Plateau, often co-occurring at riverbeds, stream margins, swampy meadows, etc. Their morphological traits differ greatly. *K. tibetica* has dense, rigid and erect culms [28]. It is also the builder species of tussocks. *B. sinocompressus* has drawf culms with brown to purplish brownleaf sheaths at the base [24]. This plant often presents an even and continuous landscape with no apparent aggregation. The growing period of both species usually ranges from May until dormancy commences in October. The flowering and fruiting phenology of these two species lasts from May to September. However, the seed germination rate of both species have been found to be low in natural condition, ranging from 0 to 13% [25, 29]. The vegetative reproduction has been reported to be ubiquitous, indicating significant importance of clonal growth in their life history [30]. Figure 1 shows the brief of the community and species.

2.2. Stand design and sample collection

All the samples were collected from one 20m×20m stand. We chose squared plot style to collect samples in order to minimize possible edge effects, as recommended by Arnaud-Haond *et al.* [31]. Some adjustment was made to facilitate sampling from tussocks and comparison of the two species. Specifically, the sampling stand were divided into 16 subplots with equal size of

5m×5m. In each subplot, we chose one tussock and scaled it to the boundary of subplot to generate the spatial coordinates (see in supplementary materials, Table S1). At each tussock, 3 randomly chosen culms of *K. tibetica* and *B. sinocompressu*s were clipped to the base respectively. Two subplots were not sampled due to the absence of *K.tibetica* tussock, so 82 plant samples were collected in total, 42 samples for each species. Community demography was investigated by setting a 50cm×50cm quadrat at each sampling tussock. The abundance, height and coverage of these two species were measured respectively. In order to compare the fine scale habitat condition for the two species, soil profiles were sampled in a parwise manner. Soil profiles for *K. tibetica* were taken within tussock while soil profiles for *B. sinocompressu*s were taken in gap between tusscoks. 5 soil profiles were made for each species. Each soil profile consists of 6 layers from surface to 1m depth at a 20cm interval. All the soil samples were analyzed for organic matter, available nitrogen, available potassium, available phosphorus, pH, electrical conductivity, saturated hydraulic conductivity and water content. The assaying procedures followed Carter and Gregorich [32]. Figure 2 demonstrates the sampling scheme.

2.3. DNA extraction and genotyping

All the plant samples were collected with caution to prevent extraneous DNA interference. After inspection of validity, samples were preserved with silica and delivered. DNA was extracted using the Plant Genomic DNA kit (Tiangen Biotech Co., Beijing, China). The 2b-RAD libraries were constructed using adaptors (50-NNN-30) to cohere the digested products as described by Wang *et al.* [33]. The sequencing was completed using an Illumina HiSeq X Ten platform. Raw reads were trimmed to remove adaptor sequences, and the 3-bp terminal positions of each read were eliminated. Reads with no restriction sites or ambiguous bases (N), low-quality positions (>20 nucleotide positions with a Phred quality score < 20), or long homopolymer regions (>8%) were discarded. High quality reads of each sample were aligned using the SOAP2 program follow the protocols by Li *et al.* [34]. A maximum of two mismatches (–v 2) were allowed for each read, and those mapped onto more than one position in the genomic reference sequence were excluded (–r 0). The match mode was set to "find the best hits" (–M 4). 41 samples of *K. tibetica* and 39 samples of *B. sinocompressu*s were successfully sequenced and the SNPs were filtered with the RADtyping program [35].

2.4. Clone assignment and spatial structure

Most of the genetic analysis below was conducted using the computer and statistical language R with various packages [36]. We acquired unique multilocus genotypes (MLGs) from the 2b-RAD genotyping. To characterize the genetic diversity and clonal structure correctly, the distinct genets should be firstly identified. The package *poppr* (version 2.6) was implemented to

assign the clonal membership [37, 38]. The main procedure consists of creating genetic distance matrix, finding the threshold and collapsing different MLGs into genets. The minimum genetic distance to distinguish different MLGs (i.e. threshold) was calculated using the *cutoff_predictor* function. Then the threshold was conveyed to the *Mlg.fliter()* function to assign the clone affiliation for each ramet. Based on the result of clone identification, the clone size, richness and distribution status were evaluated at the scale of the whole stand. Clonal richness was calculated as (G-1)/(N-1). We analyzed genotype diversity using the Shannon-Wiener index (H) and the Stoddard and Taylor's index(G). Both of the two indexes measure genotypic diversity combining richness and evenness. If all genotypes are equal in abundance, the value of G will be the number of MLGs and the value of H will be the natural log of the number of MLGs [39]. G and H were used in combination because they are complementary in weigh of abundant or rare MLGs. Evenness (E) was calculated utilizing both H and G, resulting in a ratio of the number of abundant genotypes to rare genotypes [40]. G, H and E were calculated using *diversity ()* function in package *vegan* (version 2.0) [41]. All the identified MLGs were mapped to assess the spatial arrangement of clonal patches. We performed spatial autocorrelation analysis at both ramet level and genet level follow the suggestion of Binks *et al.* [9]. The ramet level analysis included all the sampled individuals. The genet level analysis kept only one ramet per MLG. The calculation procedure was carried out using *spline.correlog()* function in *ncf* package(version 1.2)[42], with genetic distance matrix created using *dis.bitwise()* function in *poppr* and spatial coordinates generated from field records. Moran's I was calculated and 1000 resamples was implemented to find the bootstrap distribution.

2.5. Genetic diversity and evolutionary relationship

To avoid the influence of clonality on genetic diversity estimation, we removed the replicates from each genet and continued analyses using a single copy of each unique genotype. We used Genepop (version 4.7) [43] to calculate the allelic richness, expected heterozygosity, oberserved heterozygosity, as well as the inbreeding coefficents of both species at the stand scale. In order to evaluate the extent of differentiation, analysis of molecular variance (AMOVA) was implemented using *amova ()* function in *pegas* [44] package to evaluate the extent of genetic variation within and between sampling locations. The relationship between different MLGs were inspected using minimum spanning networks (MSN) with reticulation. Reticulated MSN reduces the complexity of a distance matrix and allows population structure to be more readily detectable. It is a more suitable tool than bifurcating tree for clonal organisms where many of the connections between samples are equivalent [37]. The MSN resulted was visualized using *imsn()* function in *poppr* package.

3. Results

3.1. Summary of community topology and environmental factors

The results of demography investigation showed that *B. sinocompressus* and *K.tibetica* differed greatly in abundance, height and coverage (Table S1). Although inferior to *K.tibetica* in every investigated tussock, *B.sinocompressus* appeared to be advantageous at the community scale by abundance and coverage. Gaps between *K.tibetica* tussocks were almost exclusively filled by *B.sinocompressus*. In spite of different community view, most of the soil characteristics showed no significant difference between different sampling locations (Table S2), indicating relative homogeneity of habitat condition for these two species. Detailed information about community topology and environmental factors are shown in supplement materials. Notice that clonality was not taken into consideration in the community census.

3.2. Clone assignment and spatial structure

Generally, 7710 and 21868 potential SNPs were identified for *B. sinocompressus* and *K.tibetica* respectively. The average tag number and mapping rate for *B. sinocompressus* was (4.90×10^4, 65.70%), compared with that of *K.tibetica* (5.30×10^4, 66.06%). Detailed results about sequencing could be found in supplemental materials (Table S3, Figure S1). The potential SNPs loci were filterd for further analysis if (1) more than 80% of sampled individuals could be distinguished at that locus; (2) Minor Allele Frequency (MAF) > 0.01. Finally, 41 genotyed individuals of *K.tibetica* were assigned to 23 distinct clonal linneages while the 39 genotyed individuals of *B. sinocompressus* were assigned to 21 distinct clonal linneages. Figure 3 shows the spatial arrangement of the identified clonal linneages.

The clone size and diversity results were summarized in Table 1. The clone richness was 0.53 for *K.tibetica* and 0.55 for *B. sinocompressus*. Clone size were evaluated in terms of ramet size (amount of ramets per genet) and spitial size (spatial distance between ramets of the same genet) respectively. Although the average ramet size of the two species are almost equal, the variation was much higher for *B. sinocompressus*. As for physical size, genets of *B. sinocompressus* ranged from 3.10m to 18.32m with an average of 9.85m. 42.86% (6 out of 14) of the genets of *B. sinocompressus* were found to have spread among tussocks. Ramets of MLG 10 were found to have spread over 5 tussocks. The physical size of *K.tibetica* was not available in spatial distance measure. However, it is reasonable to use the tussock size as the upper limit as our results showed all the ramets from the same genet of *K.tibetica* were restricted within tussock. Our result also showed that 50% (7 out of 14) of the *K.tibetica* were not monoclonal, indicating it's not proper to treat the whole tussock as one genetic individual. From the

perspective of spatial distribution, clonal lineages of *K.tibetica* were more evenly distributed than *B. sinocompressus*, which was consistent with the more variable size and spreading characteristic of *B. sinocompressus*.

Figure 4 showed the spatial genetic structure of both species. At the ramet level of, the Moran's I for *K.tibetica* got climax value (Y intercept 0.721,) when the distance approached zero. This correlation declined as the distance increased. At the distance of 4.70m, the correlation intercepted with the zero-correlation reference line, indicating clonality affects genetic structure no more beyond this spatial distance. The correlation value reduced (Y intercept value from 0.721 to 0.350) when no duplication of ramets were included (the genet level), reflecting the contribution of clonality to fine spatial genetic structure was significant. However, the shape of the simulated curve remained the same, which could be attributed to the clumped distribution of *K.tibetica* ramets. As for *B. sinocompressus*, the spatial genetic structure was relatively weak even at ramet level (Y intercept 0.186). Clonality affected spatial genetic structure within 14.57m, which is approximate to the biggest spatial size of the detected clones.

3.3. Genetic diversity and evolutionary relationship

7256 SNP loci were ascertained for *B. sinocompressus* while 19501 SNP loci were ascertained for *K.tibetica*, indicating a higher level of genetic variability in *K.tibetica*. (Table 2). However, the average allele number at each locus was almost the same for both species, which could be attributed to the prevalence of biallelic loci in SNP markers. Polymorphism Information Content (PIC) showed that *B. sinocompressus* had a moderate polymorphism (0.264) while *K.tibetica* had a low polymorphism (0.132). Considering that PIC value takes allele frequency into account, this result reflected that many rare alleles were preserved in *K.tibetica*. The expected heterozygosity (He) for *B. sinocompressus* was higher than that of *K.tibetica*, but the observered heterozygosity (Ho) showed the opposite trend. The inbreeding coefficients (F_{IS}) for *B. sinocompressus* was 0.559, which was six times more than that of *K.tibetica*. These result showed that the detected genetic diversity for *K.tibetica* was higher than *B. sinocompressus* and non-random mating was much common for *B. sinocompressus* which could be possibly attributed to selfing within flower or geitonogamous pollination between ramets.

The results of MSN showed contrasting pattern for the MLGs of these two species (Figure 5). The MLGs of *B. sinocompressus* were manily clustered into two groups, indicating most of the MLGs were genetically close related. The ramets assigned to specific MLG of *B. sinocompressus* could come from different tussocks. The amount of ramets per MLG were variable. Few large MLGs consists of more than 4 ramets (MLG 10). On the contrary, the MLGs of *K.tibetica* were genetically dispersed with no detected structure. The amount of ramets per

MLG were relatively stable, mainly 2-3 ramets. All the ramets assigned to specific MLG came from the same sampling location. The AMOVA results showed the origin of variance for both species. For *K.tibetica*, among locations variances contributed 78.51% to the total amount of variance while within locations variances explained the rest 21.49% proportion. For *B. sinocompressus*, most of the variances were within location (71.37%). Both species got positive *p* value at significance level of 0.05, but the effect for *K.tibetica* tended to be more significant.

4. Discussion

Alpine wetland vegetation of Qinghai-Tibet Plateau is often characterized by the prevalence of two hygrophytes: *Blysmus sinocompressus* and *Kobresia tibetica*. The latter species is a tussock builder perennial while the former one acts like a contemporary successional species during wetland deterioration. Based on SNPs identified by 2b-RAD sequencing, we contribute the first available clonal structure of these two species and the fine scale genetic structure that could help to understand the process of degrading succession in alpine tussock swamp.

4.1 Clonal structure and spatial pattern

Size and spatial arrangement of genets are of fundamental importance in clonal population as they affect the mating opportunities of individuals and provide the basis for long-term preserving and expanding [10, 11, 13]. Our results have confirmed *K.tibetica* has a phalanx growth form. The clonal diversity of *K.tibetica* (R=0.53, H=3.02) is similar to *K. pygmea* (R=0.41, H=3.02) [23]. All the ramets of a specific clone are restricted within tussock, which reflects the production of short rhizome described in previous studies [26]. This phalanx growth form is also supported by the steep autocorrelation curve (Figure 4) which shows the spatial range limit of clonality on genetic structure is 4.70m. However, about 50% (7 out of 14) tussocks resides more than one MLG, indicating that multiple clone may coexist in one tussock. It may be attributed to the seedlings recruitment from the seedbank, as tussocks tends to promote the sexual reproduction of inhabitant species [15, 45, 46]. Our results also imply that it may be incorrect to treat all the individuals from a clumped cluster as belonging to one clone, which is consistent with the findings in *Carex sempervirens*[18].

As for *B. sinocompressus*, the population tends to be constituted by intermingled genets with guerilla growth form. The detected largest clone size is 18.32m (Table 1) which complies with the record of Hu *et al.* [25] as a far creeping species. The results of AMOVA also show that the major part of variation is distributed within tussock for *B. sinocompressus* (Table 3), which is in accordance with the expanding of genets among different tussocks (Table 1). However, the clone size distribution is uneven. The fluctuation in clone size is greater for *sinocompressus* than *K.tibetica* (Table.1, Figure 3). There are two causes for a large variability in clone size: (1)

Clones with different sizes may reflect successive events of seedling recruitment ranging from old and large genets to recently established, small genets; (2) small clones could also represent remains of formerly larger clones that partly died [23]. Considering the recent emergence of *B. sinocompressus* in the succession, the first explanation is plausible. Additionally, *B. sinocompressus* has similar clone richness but lower diversity (R=0.55) compared with *K.tibetica*, indicating sexual reproduction may be nearly equal in both species. These results in combination imply that clonal growth in guerilla form may enhance the clone expansion and consolidate the advantage of *B. sinocompressus* in the community, which is a strategy favored by clonal plants in optimal environment [47].

4.1 Mating patterns and succession process

Although clonal growth could increase the probability of within-clone movement of gametes that may lead to fitness cost (e.g., self-fertilized offsprings), some researches have shown that this effect is contextual on the interaction between the spatial arrangement of clones and biological traits [12, 13]. Both species are wind pollinated and have mixed reproductive modes, but they have contrasting inbreeding level (Table 2). The low inbreeding level in *K.tibetica* implies: (1) the pollen flow among tucssocks of *K.tibetica* is not hindered by the spatial separation and (2) geitonogamous selfing within tussock is effectively avoided. The mechanism for inbreeding avoidance appears to be complex. One possible reason is the dichogamous flower development (dichogamy). It's reported that synchronization of sex function among ramets of a clone (i.e. ramets of the same clone present the same sexual phase at a given time) could limit inter-ramet geitonogamy[48]. Cruden has reported the prevalence of this phenomenon from 37 diverse angiosperm families, including many rhizomatous clonal perennials (e.g., *Typha*, *Sparganium*, *Scirpus*) [49]. Altenatively, self-compatibility or postzygotic barriers may also contribute to the inhibition of inbreeding [9]. Further research effort is needed as the information about breeding sytem of *Kobresia* is very limited. The higher inbreeding level of *B.sinocompressus* could be explained by the effect of clone expansion. As the clone size gets larger, it becomes more difficult for outcrossing pollen to disperse across different clones. The low height of *B.sinocompressus* may also contribute to the difficulty because the winds tend to be weaken in lower surface of microtopography[50]. Our results show that phalanx growth form is not necessarily prone to inbreeding. The effect of clonal structure on mating pattern tends to be contextual on both biotic and abiotic factors.

Many plants utilize a combination of sexual and asexual reproduction and the balance between these strategies varies widely within and among taxa [51]. Facultative sexual reproduction in clonal plants plays an important role in maintaining the genetic diversity and evolutionary potential. Thus, the genetic relatedness of original genets could influence the

population viability in long term. In wetlands, the "opportunity window" for succession often turns out when flood retreats and seedlings emerge rapidly due to dormancy relief [52, 53]. It is also the case for *B.sinocompressus* which colonizes the gaps between *K.tibetica* tussocks as the retained water disappears. However, most genets of *B.sinocompressus* are more closely related and assigned to two clusters (Figure 5), indicating that they are prone to be inbreeded offsprings of few old genets. Consequently, the evolutionary potential is constrained by the ancestral genets, resulting in deficiency of genetic diversity. Zhao *et al.* have found that input of genets from seedlings matters in determining the genetic diversity for clonal plants [26], which is consistent with our results. Although the combination effect of clonal growth and seedlings enable the temporary successional advantages, *B.sinocompressus* may be vulnerable to future disturbance, such as grazing and degradation [25, 54]. On the contrary, genets of *K.tibetica* are more evolutionary separated and present a high level of variability. Previous studies have suggested that even low rates of seedling recruitment are sufficient in maintaining high levels of genetic diversity [9, 10]. As isolation among tussocks tends to be enhanced during degradation, the coexistence of genetically distant genets within tussock is of vital importance in providing the necessary levels of gene flow. Generally, our result supports the view that the genetic load of original genets explains the high genetic diversity of *Kobresia*. The remaining tussocks in degrading wetlands stands as valuable genetic relics of the former well-developed *K.tibetica* meadow, which is worthy of more conservation or restoration attention.

5. Conclusions

In summary, we reveal the clonal structure and fine scale genetic structure of two alpine plants (*Kobresia tibetica* and *Blysmus sinocompressus*) on the context of wetland succession. The tussock builder *K.tibetica* has a phalanx growth form but different genets could coexist within the same tussock. It's not proper to treat tussock as one genetic individual. The *B. sinocompressus* has guerilla growth form and considerable variability in clone size, indicating a successive recruitment from seedlings. Our results demonstrate the combination of clonal growth and seedlings contribute to the advantage of *B. sinocompressus* at the early stage of degradation. Nevertheless, most genets of *B. sinocompressus* tends to be inbreeded offsprings of few old genets, resulting in deficient evolutionary potential. On the contrary, genets *K.tibetica* present inbreeding avoidance despite of more closely placement of ramets, indicating tussocks are valuable genetic relics worthy of conservation attention. It is important to recognize that this study only assessed one community in fine scale, and the underlying mechanism is not clear due to the lack of information on inbreeding system of both species. Further research effort is needed to unfold the gene flow process of both species in various habitat condition, especially with the knowledge of pollination biology and degrees of self-compatibility.

Supplementary Materials: Table S1: Community demography showing the abundance, coverage and height of both species; Table S2: *t*-test results of eight types of soil properties demonstrating the difference in the microhabitat; Table S3 Summary of results of enzyme digestion and mapping for each sampled individuals; Figure S1: the overall sequencing quality showing the distribution and quality of acquired bases.

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Table 1(on next page)

The clone size and diversity information of both species

N, number of samples; G_g, number of genets; R, genotypic richness; H, Shannon-Wiener index; G_s, Stoddard and Taylor index; E, evenness index; N_{mon}, number of monoclonal tussock; N_{mul}, number of multiple-clonal tussock; N_{sp}, number of clones spreading over tussocks. Ramet size is the amount of ramets per genet; spatial size is the spatial distance between ramets of the same genet. * indicates spatial size of Kob is not available because all ramets reside within tussock.

1

Species		Bly	Kob
Richness	N	39	41
	G_g	21	23
	R	0.55	0.53
Ramet size	min	1	1
	max	8	3
	mean(se)	1.86(0.37)	1.78(0.19)
Spatial size(m)	min	3.10	*
	max	18.32	*
	mean(se)	9.854(0.96)	*
Diversity	G_s	11.61	18.47
	H	2.76	3.02
	E	0.72	0.90
Distribution	N_{mon}	1	7
	N_{mul}	13	7
	N_{sp}	6	0

Table 2(on next page)

Summary of the genetic diversity information and inbreeding levels for both species

n, number of loci; N_A, average allele number per loci with SD in parenthesis; PIC, average polymorphism information content; H_e, expected heterozygosity; H_o, observered heterozygosity; F_{IS}, inbreeding coefficients.

1

Species		loci information		heterozygosity		inbreeding
	n	N_A	PIC	He	Ho	F_{IS}
Bly	7256	2.063(0.242)	0.264(0.145)	0.338	0.081	0.559
Kob	19501	2.023(0.207)	0.132(0.108)	0.153	0.143	0.093

Table 3(on next page)

Summary of analysis of molecular variance (AMOVA) of both species showing the origin of variances

1

Species	Source	df	SSD	MSD	Variance	% total	*p*
Kob	Among tussocks	13	80904.69	6223.44	1944.52	78.51	<0.001
	Within tussock	27	14368.17	532.15	532.15	21.49	
	Total	40	95272.85	2381.82			
Bly	Among tussocks	13	40434.1	3110.32	589.75	28.63	0.048
	Within tussock	25	36754.33	1470.17	1470.17	71.37	
	Total	38	77188.44	2031.28			

Figure 1

Species and community landscape

(a) *Blysmus sinocompressus*. (b) community view with blue circle indicating *B. sinocompressus* and red circle indicating *K. tibetica*. (c) *Kobresia tibetica*

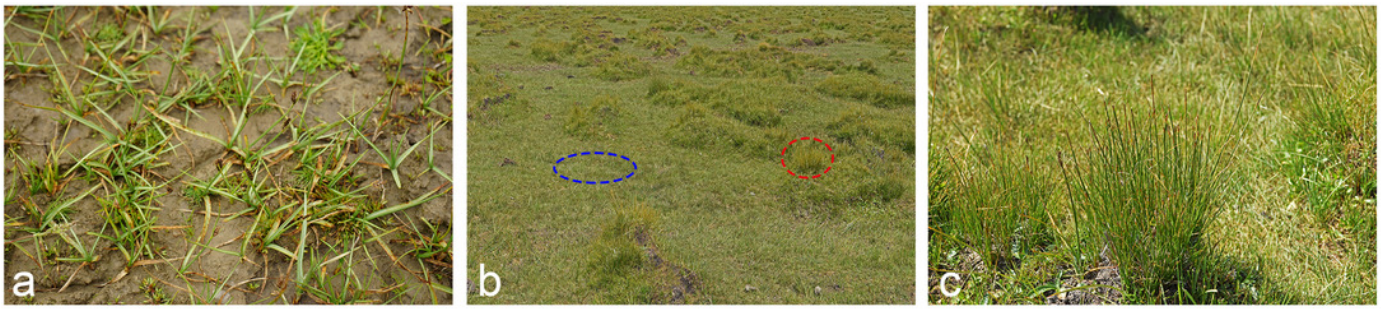

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Figure 2

Stand design and sampling scheme

The solid circles with number represent the sampled tussocks. The dashed circle indicates where the soil profile was taken. The inset shows 3 samples of *K. tibetica* and 3 samples of *B. sinocompressus* were taken respectively at each tussock.

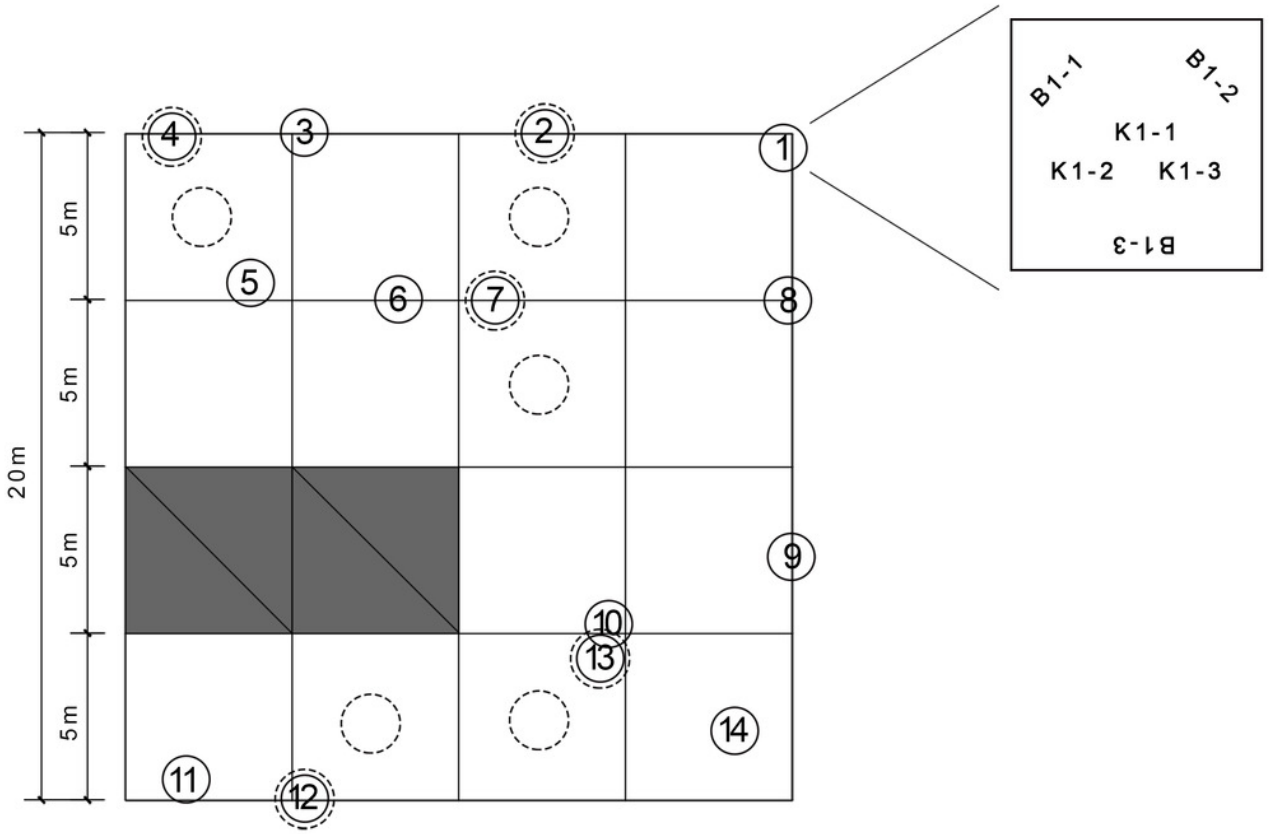

Figure 3

The spatial arrangement of detected clonal linneages

Each sample were plotted according to their clonal assignment and the spatial position. The same symbol indicates the same clonal membership. Notice symbols between species are not relevant. Bly is short for *Blysmus sinocompressus*. Kob is short for *Kobresia tibetica*. The same hereafter.

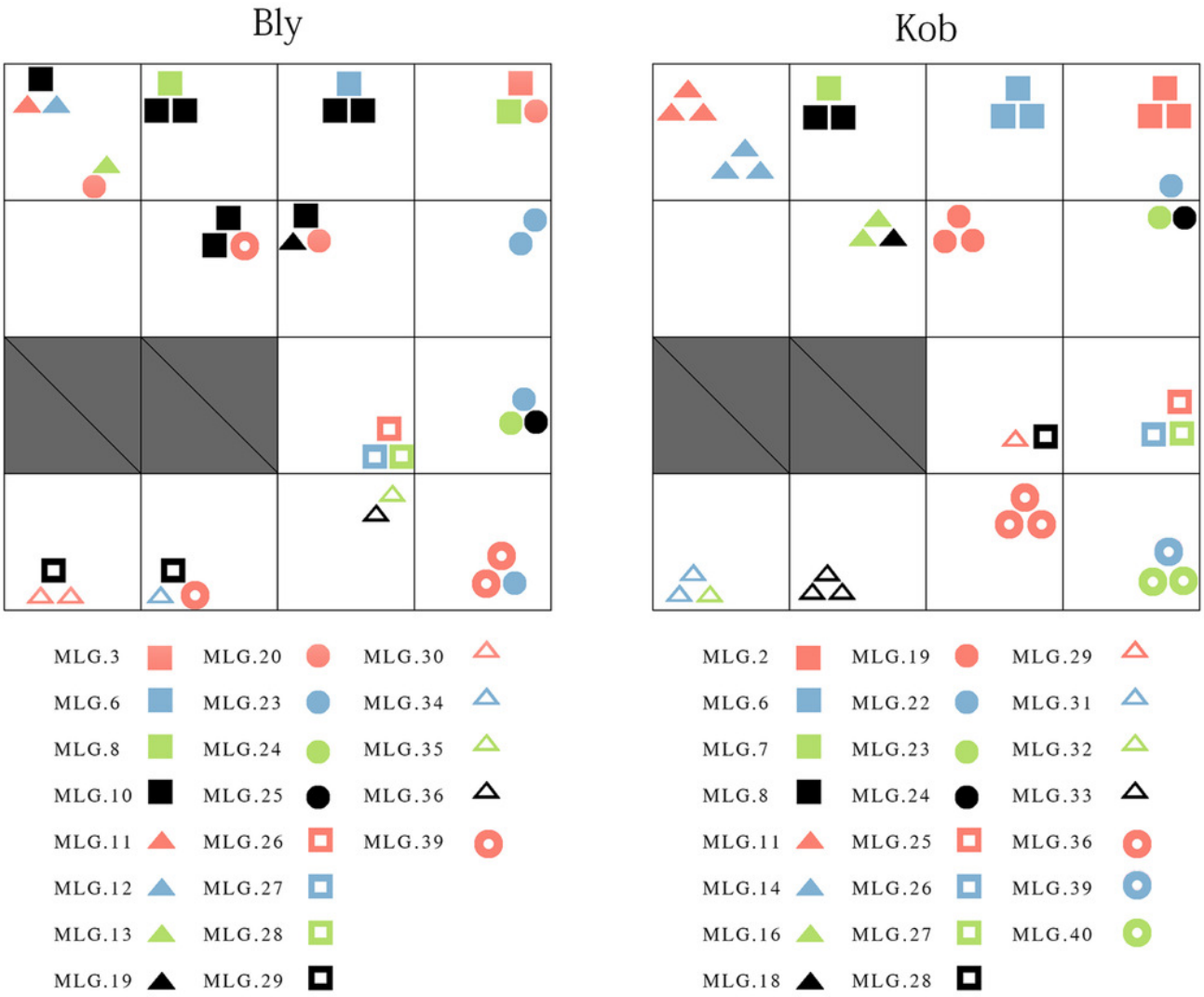

Figure 4

Spatial autocorrelation between kinship and geographic distance for both species at ramet and genet levels

The dashed blue line envelopes the bootstrap distribution at 1000 resamples. The red line points out the position of intercept with zero-reference line beyond which genetic relationship are no more similar than that expected by chance alone.

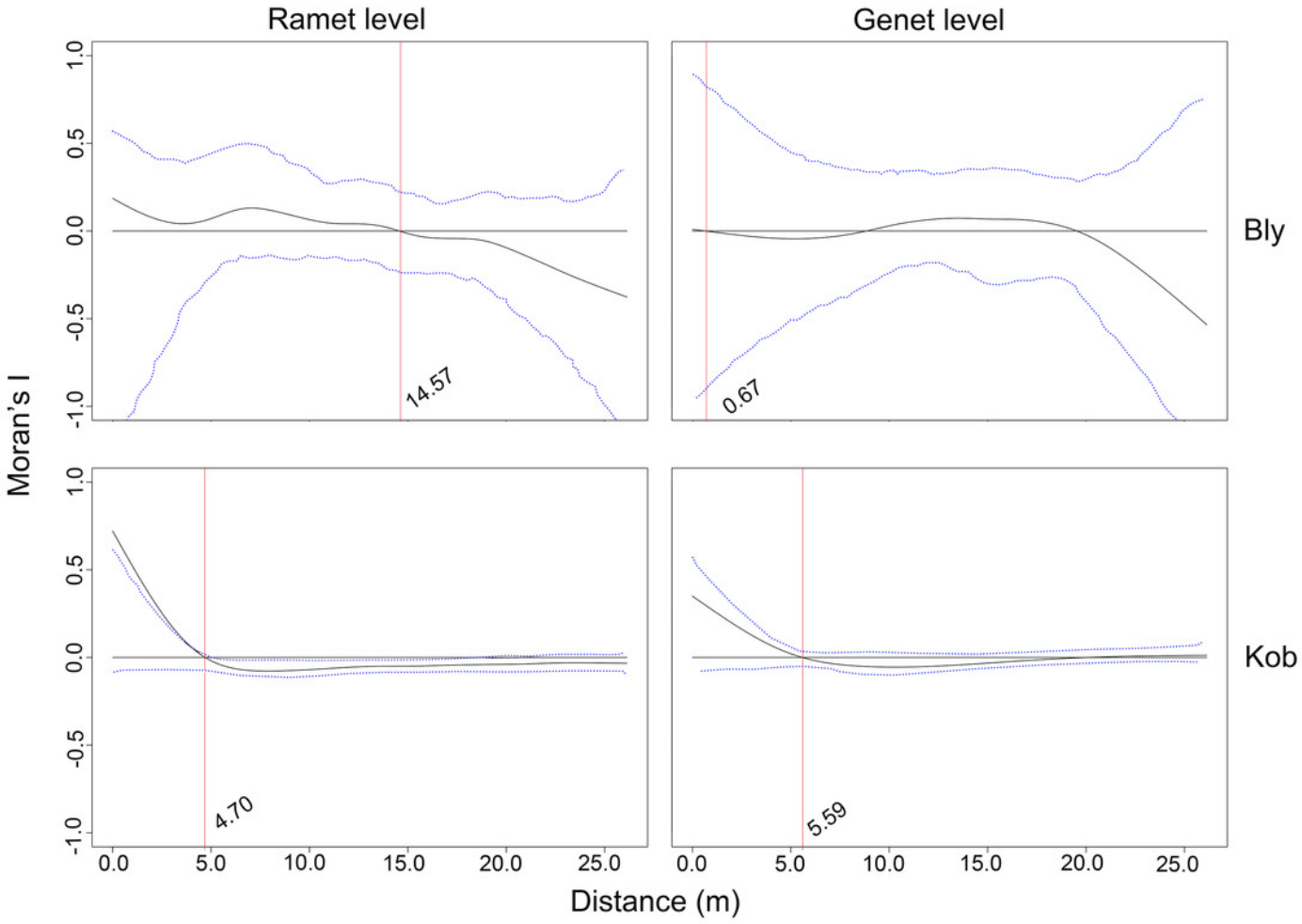

Figure 5

Minimum Spanning Tree (MSN) showing the evolutionary relationship of MLGs

Size of node is proportional to the amount of assigned ramets. Color represents the tussock where the samples were taken. Wider and darker line indicates relatively higher relatedness. The position of nodes is arbitrary.