Markers associated with altitude and ecological factors in hexaploid *Campeiostrachys nutans* (Griseb.) J. L. Yang, B. R. Baum et C. Yen and tetraploid *Roegneria nutans* (Keng) Keng

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Abstract The genetic basis of adaptation can be unraveled directly at the genome level, without regard to selectively advantageous genes or traits. Genetic variation and adaptation of polyploidy in the evolution of plants is becoming an area of intense interest. Whether hexaploid wheatgrass species has higher adaptability to altitude than tetraploid or not needs to be proofed by population genomic data. A fluorescence-based AFLP technique was used to investigate the allele share of hexaploid *Campeiostrachys nutans* (Griseb.) J. L. Yang, B. R. Baum et C. Yen and tetraploid *Roegneria nutans* (Keng) Keng as well as the genetic variation and natural selection in 22 natural populations and their association with ecological factors. Of all the AFLP bands, 84.29 % were found in common between two different ploidy wheatgrasses. In addition, we found that hexaploid *C. nutans* had 1.45 times the allelic frequency and more large-sized bands than tetraploid *R. nutans*. After genetic analyses, the next factor examined should be the effect of altitude on genetic variation. There were no significant inter-population genetic differentiations, suggesting that distance did not contribute to spatial isolation. Altitude and soil nutrient availability might play an important role in maintaining the genetic diversity. A

certain percentage of positive selection loci were discovered in total genome allele. Hexaploid wheatgrass with higher ploidy numbers can show strong genetic adaptability to adverse high-altitude condition because of its complex genomic background with an additional H genome, which supports our hypothesis. Though this evolutionary process is believed to be the driving force behind sympatric speciation, we cannot forecast that hexaploid wheatgrass species will diversify into two species in the long term, but believe that diversification under local selection helps to increase the adaptability to the changes in altitude.

Keywords Allelic share · *Campeiostrachys nutans* · Environment · Genetic variation · Polyploid · *Roegneria nutans* · Selection

Introduction

Polyploidization is seen as one of the major processes that has driven and shaped the evolution of higher organisms (Stebbins 1950; Grant 1981). A common assumption is that populations with higher ploidy numbers have higher levels of genetic diversity (Mallet 2007; García-Verdugo et al. 2009), especially if they are allopolyploids and are not of recent origin (Ramsey and Schemske 2002; Luttikhuisen et al. 2007). This assumption has been tested in some population genetic studies that analyzed two (tetraploids and diploids in Luttikhuisen et al. 2007; Abbott et al. 2007) or three

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(García-Verdugo et al. 2009) ploidy levels. Interestingly, opposite findings of lower (Kim et al. 2009) or higher (García-Verdugo et al. 2009) genetic diversity in higher polyploid plants has led to debate about the benefits of polyploidy with regard to genetic diversity. Polyploid evolution independent of the plants' progenitors and habitat preference is a possible explanation for higher diversity and lower polyploidy. In addition to contributing to the genetic diversity of multiploid species, studying polyploidy led to a recent discovery regarding the contribution of polyploidy to the response of species and communities to environmental change (Davis et al. 2005). Genetic variation in a population can be induced and maintained by the force of natural selection in heterogeneous habitats (Zhang 2004). Altitude can also affect the genetic variation and lead to rapid evolution in alpine plant species (Geng et al. 2009). Plant populations rapidly diverge in response to changes in soil conditions (Snaydon and Davies 1982), and a population's genetic variation is related to the level of soil nutrients (Orians et al. 2003). At the same time, soil and eco-environmental conditions have a notable effect on a population's genetic structure along altitudinal gradients (Lazrek et al. 2009). Based on the AFLP molecular marker, genome scans provide a means to identify the genetic basis of such adaptations to adverse environmental conditions such as high altitude without previous knowledge about the particular genetic variants or traits under selection (Fischer et al. 2011).

Allopolyploid *Campeiostrachys nutans* (Griseb.) J. L. Yang, B. R. Baum et C. Yen ($2n = 6x = 42$, **StStYYHH**) and *Roegneria nutans* (Keng) Keng, ($2n = 4x = 28$, **StStYY**) are very similar in their morphology. The characteristics that best distinguish these two species are chromosome number and genomic constitution (Löve 1984). Evidence from different studies suggest that polyploidy is not an evolutionary dead end (Otto and Whitton 2000), but it does not prove that polyploidization contributes to evolutionary success (In review of Otto 2007). It is often thought that polyploids have an advantage over diploids via their greater ability to mask deleterious mutations. Although the evolutionary success of polyploid plants has been widely recognized, there is virtually no information on how some related polyploid with similar genomes have evolved after their formation. Whether polyploidization increases the fitness and adaptation of a group relative to taxa that have undergone no or less

polyploidization is a question that has yet not to be answered. Facing the same selective pressures, whether higher polyploidy Triticeae species may well have undergone beneficial evolutionary transitions? What kind of genetic diversity do different polyploidy Triticeae species made up of **St** and **Y** genomes show, and which species shows more genetic variation after examination of a multi-population neutral allele and selection loci by the altitude? No study has examined genetic variation and its association with ecological factors in context with the population genetic evidence of the two Triticeae species. Can these genetic divergences be attributed to the soil change along altitudinal gradients? The answer to these questions will help us to understand the evolutionary history of polyploidy and facilitate the genetic conservation of two important Triticeae species on the Qinghai-Tibetan Plateau. After we determined the genetic diversity and differentiation at population level from the standpoints of germplasm conservation of two Triticeae species (Yan et al. 2009; Yan et al. 2010), the specific objectives of this investigation are to explore the consanguinity between hexaploid *C. nutans* and tetraploid *R. nutans*, to calculate the genetic relationship at the population level between altitude, soil characters and population genetic indexes, and to test the selection loci under the altitude from the view of genetic basis for the ecological adaptation.

Materials and methods

Plant materials and soil sampling

Seed samples from 14 populations of *C. nutans* (Griseb.) J. L. Yang, B. R. Baum et C. Yen ($2n = 6x = 42$, **StStYYHH**) and 8 populations of *Roegneria nutans* (Keng) Keng, ($2n = 4x = 28$, **StStYY**) were collected in Maqin County, Qinghai Province, China. The geographical conditions of the collecting sites are presented in Table 1. Seed samples were collected following the method used in Jin and Lu (2003). Approximately 30 individuals from each population were germinated, and seeds from each spike were planted in a plastic cup that was placed in a greenhouse. After 5–6 weeks, approximately 1 g of pooled leaf material from 10 seedlings of each spike was harvested, placed in a zip-lock plastic bag, and stored in a freezer (-80°C) until used. Culture

Table 1 Geographical condition of sampled populations of two wheatgrasses from the Qinghai-Tibetan Plateau of China

Species	Population identity	Altitude (m)	Sample size	Latitude (N)	Longitude (E)
<i>C. nutans</i>	E4	2,800	30	34°31'32"	100°30'11"
	E5	2,940	29	34°30'56"	100°29'18"
	E14	3,060	22	34°31'36"	100°29'23"
	E15	3,080	22	34°31'48"	100°30'11"
	E3	3,200	28	34°31'21"	100°28'12"
	E13	3,260	21	34°31'1"	100°26'47"
	E2	3,400	28	34°31'52"	100°29'17"
	E1	3,540	26	34°31'39"	100°27'51"
	E11	3,680	26	34°30'57"	100°25'56"
	E12	3,780	21	34°29'57"	100°24'14"
	E9	3,920	27	34°30'34"	100°24'46"
	E6	3,940	30	34°30'47"	100°23'58"
	E10	3,950	30	34°30'43"	100°25'25"
	E8	4,020	19	34°30'21"	100°23'53"
<i>R. nutans</i>	R5	2,940	24	34°30'56"	100°29'18"
	R3	3,200	28	34°31'21"	100°28'12"
	R2	3,400	29	34°31'52"	100°29'17"
	R1	3,540	18	34°31'39"	100°27'51"
	R11	3,680	27	34°30'57"	100°25'56"
	R9	3,920	28	34°30'34"	100°24'46"
	R6	3,940	31	34°30'47"	100°23'58"
	R7	4,040	31	34°30'51"	100°24'41"

samples were deposited in the Institute of Biodiversity Science, Fudan University in Shanghai. Soil from an underground depth of 0–20 cm was sampled at 15 sites using a stainless steel soil-sampling probe in August of 2005, and three replicates from each site were taken. After disposing of the pebbles and other wastes, air-dried soil samples were placed in zip-lock plastic bags and stored in a freezer (−80 °C) until used.

Fluorescence-based AFLP technique

A fluorescence-based AFLP (amplified restricted fragment length polymorphisms) technique was used to investigate the genetic variation of 22 natural populations of two wheatgrasses from the Qinghai-Tibetan Plateau at different altitudes. Five selected AFLP primer combinations (E-AAC/M-CAA, E-AAC/M-CTT, E-ACG/M-CTT, E-AGG/M-CAG, and E-ACG/M-CTG) were used in this study. A detailed procedure for the fluorescence-based AFLP was described in the publication of Yan et al. (2010), including DNA extraction, digestion, ligation, PCR amplification, electrophoresis and scoring.

Allele frequency inferences based on higher probability of belonging to one ancestral species

We used Structure v.2.2 (downloaded from <http://pritch.bsd.uchicago.edu>) and applied the method of examining dominant markers, such as AFLP, with regard to polyploidy, as described in detail by Falush et al. (2007). To explore the allelic share of two Triticeae species with similar genomic constitutions and infer the contribution of the H genome to genetic differentiation, we adopted the admixture model because it is the most appropriate model for dealing with introgressed populations; it allows for the analysis of admixture and the correlated allelic frequencies (Pritchard et al. 2000a). We used prior information about the populations to assist in clustering and obtained information about the distribution of Q (the estimated membership coefficient) for each individual as well as estimating the mean. Based on a higher Q and a posterior probability, the allelic frequency for each individual was selected for the two wheatgrasses (Pritchard et al. 2000b).

Principal coordinate analysis of all individuals of two Triticeae species

In order to analyze the genetic relationship of two Triticeae species based on the individual allelic data, the genetic dissimilarities were calculated based on Jaccard's coefficients (1908) between individuals after our former population-level calculation of each species (Yan et al. 2009) using the SIMQUAL option, and they were ordered in a dissimilarity matrix. Genetic dissimilarity (DS) was calculated as $DS = 1 - \text{Jaccard's similarity}$. Principal coordinate analysis (PCoA) was performed using the DCENTER and EIGEN programs, as described by Gower (1996) in NTSYS v.2.1. This multivariate approach was chosen to complement the cluster analysis because the analysis is more sensitive to closely related individuals, whereas PCoA is more informative regarding distances among major groups (Hauser and Crovello 1982).

Detection of non-neutral loci

We used the software BayeScan V.2.01 to identify candidate loci under natural selection from genetic data, using differences in allele frequencies between populations based on the multinomial-Dirichlet model following the instruction of Foll and Gaggiotti (2008). Selection is introduced by decomposing F_{st} coefficients into a population-specific component (beta) shared by all loci, and a locus-specific component (alpha) shared by all the populations using a logistic regression. Departure from neutrality at a given locus is assumed when the locus-specific component is necessary to explain the observed pattern of diversity (alpha significantly different from 0). In this study, three Jeffrey's scales of evidence were defined as "strong selection loci" with 90.9 % confidence corresponding to $\text{Log}_{10}(\text{BF}) = 1.0$ (BF, Bayesian factor), "very strong selection loci" with 96.9 % confidence corresponding to $\text{Log}_{10}(\text{BF}) = 1.5$, and "decisive selection loci" with 99 % confidence corresponding to $\text{Log}_{10}(\text{BF}) = 2.0$. A positive value of alpha suggests diversifying selection, whereas negative values suggest balancing or purifying selection. In our genome scan, we used a threshold for the $\text{Log}_{10}(\text{BF}) = 1.0$ (strong) for a marker to be considered under selection. This corresponds to a posterior probability greater than 90.9 % for the model accounting for selection.

Correlation analyses between genetic indices and other environmental parameters

The analytical methods and results of soil parameters were described in detail in our publication (Wang et al. 2009). Two methods were used to test the association between genetic indices and other environmental parameters. First, the correlation between the genetic distances and the corresponding geographical origins of the populations, such as altitude, latitude and longitude, were investigated by the Mantel test (Mantel 1967). Second, both the Pearson and Spearman correlation coefficients between the intra-population neutral genetic diversity (calculated on the software Popgen32 excluding the selection loci) and the other environmental parameters were calculated using the bivariate test in SPSS 11.5 for Windows. All curve regression models were then used to test the correlations between the genetic diversity of the populations and other explanatory factors. The probabilities for the significance of all correlation coefficients were determined followed by a Mann–Whitney U test (adjusting the α -level by Bonferroni inequality). The analysis were performed using the program SPSS.

Results

Identification of alleles shared by hexaploid

C. nutans and tetraploid *R. nutans*

Although polymorphisms among the populations of the two species were detected for the majority of fragments, only 210 clear and consistent fragments were used for further genetic evaluation. The overall percentage of polymorphic AFLP loci was 73.52 % for *C. nutans* and 64.13 % for *R. nutans*, suggesting that more alleles are likely to be homozygous within the tetraploid *R. nutans* populations compared to the hexaploid *C. nutans* populations. Bayesian cluster analysis of the two different polyploid wheatgrass species indicated that 84.29 % of all *C. nutans* and *R. nutans* alleles were consanguineous. The remaining unshared alleles were rarely found in the tetraploid *R. nutans*; however, the shared alleles were commonly detected in hexaploid *C. nutans* (Fig. 1). The frequency of each allele in hexaploid *C. nutans* was 1.45 times that of tetraploid *R. nutans*, on average. These

results demonstrate that the tetraploid species *R. nutans* contains only a few rare alleles that are commonly present in the hexaploid species *C. nutans*. Moreover, longer bands (alleles) were more commonly found in hexaploid *C. nutans* for each primer combination (Fig. 2).

Principal coordinate analysis (PCoA) of all individuals from two wheatgrass species

We examined all individuals from two Triticeae species using principal coordinate analysis (PCoA), which reduces multivariate data sets into trends of maximum relevance. Axes 1 and 2 accounted for 36 and 23 % of the variance, respectively (Fig. 3). Populations of each species formed clusters, but species were not separated by either axes. Populations from similar altitudes and geographical origins did cluster by species. The variance from the tetraploid *R. nutans* individuals was mainly distributed in the first axis, while the variance for the more highly diversified hexaploid *C. nutans* individuals was mainly distributed along both the axes. Tetraploid and hexaploid individuals from the Triticeae species overlap at the approximate center of the two axes.

Selection loci between two polyploidy Triticeae species

Candidate selection loci in two Triticeae species were examined (Fig. 4). Within each species, a certain percentage of selection loci were discovered. Hexaploid *C. nutans* showed a higher percentage of strong

Fig. 2 Relationships between the allele frequency ratio between tetraploid *R. nutans* and hexaploid *C. nutans* and the length of each allele. The *x*-axis represents the length of each allele, while the *y*-axis shows the corresponding allele frequency ratio between *R. nutans* and *C. nutans*

selection loci compared to tetraploid *R. nutans* at three Bayesian factor thresholds (Table 2). A total of 10 loci with the positive Bayesian factor ($\text{Log}_{10}(\text{BF}) > 0$) showed the diversifying selection against two wheatgrass species under the different altitude condition. Most of selection loci showed a larger F_{st} value under local selection, while minor of selection are expected to show a lower F_{st} value loci under balancing selection or purifying selection (Fig. 4).

Genetic variation and structure of wheatgrass populations associated with altitude and soil conditions

The sampled *C. nutans* and *R. nutans* populations covered a wide range of altitudes (1,200 m) and a

Fig. 1 Comparisons of the allele frequency between tetraploid *R. nutans* and hexaploid *C. nutans*. The *x*-axis represents the frequency of an allele in tetraploid *R. nutans*, while the *y*-axis shows the frequency of the same allele in hexaploid *C. nutans*

Fig. 3 PCoA analysis of 601 individuals sampled from 22 populations of two Triticeae species. The percentage of the variance explained by each axis is given in parentheses.

Individual samples of *R. nutans* are circled by straight lines and by dotted lines for *C. nutans*

smaller range of latitudes (less than 30 km horizontally). After correcting with the Mantel test, there was no significant relationship between the inter-population genetic distances and their corresponding geographical sites for *C. nutans* ($r = 0.1108$, $P = 0.7944$ for altitude; $r = 0.0471$, $P = 0.6424$ for latitude; $r = 0.2519$, $P = 0.9032$ for longitude; and $r = 0.1595$, $P = 0.9188$ for both latitude and longitude) or for *R. nutans* ($r = 0.2839$, $P = 0.911$ for altitude; $r = 0.1099$, $P = 0.7115$ for latitude; $r = 0.1295$, $P = 0.7733$ for longitude; and $r = 0.0825$, $P = 0.4696$ for both latitude and longitude).

The regression analyses applied here demonstrate a significant relationship between the intra-population neutral genetic diversity and the altitude change for *C. nutans* populations (correlation coefficients: $R^2 = 0.6963$, $P = 0.0026$ for H_e and $R^2 = 0.6838$, $P = 0.0031$ for I) and for the *R. nutans* populations (correlation coefficients: $R^2 = 0.5089$, $P = 0.1013$ for H_e and $R^2 = 0.5693$, $P = 0.0531$ for I), suggesting an effect from altitude on the intra-population genetic diversity of the two Triticeae species (Table 3). No significant relationship was found between the altitude and the chemical contents of the soil and the polymorphic percentage of the two species, except for potassium, calcium and magnesium in *R. nutans* (Table 3). Of the nine soil chemical indexes, nitrogen and magnesium content showed significant (or not) effect on intra-population neutral genetic diversity (H_e and I) in both Triticeae species along the altitudinal gradient.

Discussion

Allelic ancestry and evolutionary comparison between hexaploid *C. nutans* and tetraploid *R. nutans*

In this study, we found a greater number of shared alleles and a high allelic frequency in hexaploid *C. nutans* (StStYYHH) compared to tetraploid *R. nutans* (StStYY). PCoA analysis also indicated a higher genetic variability in hexaploid *C. nutans* than tetraploid *R. nutans*, which is in agreement with our original hypothesis. In the *Roegneria* genus, the **Y** genome may have a similar origin and evolutionary history to the **St** genome (Lu and Salomon 2004; Liu et al. 2006). Different hypotheses regarding the origin of **Y** genome have been suggested, and the origin is still under debate (reviewed in Sun and Salomon 2009; Mason-Gamer et al. 2010). Regardless of the origin of **Y** genome, it appears that the addition of an **H** genome brings complexity to the entire genetic pool of hexaploid *C. nutans*. We deduce that it is the **H** genome that leads to differences in the allele frequency and diversity of the two different polyploidy Triticeae species. In a related study, hexaploid bentgrasses (*Agrostis gigantea*) were found to be genetically distinct from the tetraploid *A. palustris* and have more species-specific markers through the use of AFLPs (Vergara and Bughrara 2004). These findings agree with our hypothesis of higher genetic variability

Fig. 4 Bayesian test for selection on individual AFLPs. Approach was implemented in BayeScan for the **a** Hexaploid *C. nutans* and **b** Tetraploid *R. nutans* separately. F_{st} is plotted against the $\log_{10}$ of the posterior odds (PO). The vertical line

shows the critical PO used for identifying outlier markers. The markers on the right side of the vertical line are candidates for being under positive selection

Table 2 Selection loci and their percentage of two different polyploidy Triticeae species

Species	Strong selection loci	Very strong selection loci	Decisive selection loci
Tetraploid <i>R. nutans</i>	17 (8.1 %)	9 (4.3 %)	4 (1.9 %)
Hexaploid <i>C. nutans</i>	31 (14.8 %)	22 (10.5 %)	15 (7.1 %)

and allele diversity in hexaploid *C. nutans* than in tetraploid *R. nutans*. A slightly greater allelic diversity per locus and a higher allelic frequency was also

detected in hexaploid *Triticum aestivum* compared to its tetraploid species ancestor (Alamerew et al. 2004). Although hexaploid *C. nutans* displayed more AFLP variability compared to *R. nutans*, both species are genetically diverse and widely distributed across a diverse range of altitudes on the Qinghai-Tibetan Plateau. *C. nutans* is the dominant species at high, cold pasture lands (Chen and Jia 2002) and was found to have a higher degree of genetic variability in this study. The hexaploid *C. nutans* species is highly evolved and is indigenous to the high, cold rangeland of the Qinghai-Tibetan Plateau (Wang et al. 2006). Similar situations are also commonly found in other

Table 3 Correlation coefficients between the altitude, the soil characteristics and the population genetic indexes of two Triticeae species

	Altitude	Latitude	Longitude	pH	Organic matter	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)	Sodium (Na)
<i>C. nutans</i>												
P	-0.190	0.186	0.111	-0.337	0.042	-0.058	0.164	-0.123	0.062	0.430	0.219	-0.453
He	0.6963 ^a	0.478	0.282	0.341	-0.122	-0.610 ^a	0.147	-0.394	0.375	0.532 ^a	-0.403	0.119
I	0.6838 ^a	0.468	0.271	0.313	-0.105	-0.604 ^a	0.152	-0.396	0.366	0.541 ^a	-0.401	0.089
<i>R. nutans</i>												
P	-0.047	-0.759 ^a	-0.470	-0.253	0.191	-0.051	-0.180	-0.587 ^a	0.663 ^a	0.544 ^a	0.204	-0.028
He	0.5089 ^a	-0.199	-0.732 ^a	0.208	-0.132	-0.437 ^a	-0.085	-0.858 ^a	0.801 ^a	0.669 ^a	-0.059	0.042
I	0.5693 ^a	-0.214	-0.746 ^a	0.231	-0.156	-0.449 ^a	-0.105	-0.846 ^a	0.813 ^a	0.644 ^a	-0.083	0.052

^a Correlation is significant ($P < 0.05$, 2-tailed test)

polyploid plants (e.g., Monte et al. 2001; Ohsawa et al. 2007; García-Verdugo et al. 2009). The findings regarding two wheatgrasses indicate that a high degree of polyploidy can provide a strong resistance to adverse conditions, which supports the hypothesis that polyploidy, is a driving force in the evolution of plant species (Stebbins 1950). The Qinghai-Tibetan Plateau represents an important geographical landscape and pasture region in China and is where the Huanghe River, Yangzi River and Lanchang River originate. The hexaploid *C. nutans* species should be studied in greater detail from the perspective of germplasm conservation, ecological importance and the grazing implications on animal husbandry.

Evolutionary process and speciation fate of two polyploidy wheatgrass species

Positive selection acting on a locus increases population divergence, whereas balancing selection keeps it low (Lewontin and Krakauer 1973). Similar forces of selection and evolution experienced by two polyploid species may cause different diversifying and stabilizing selection on the hexaploid and tetraploid wheatgrass species. Loci under local selection are expected to show a larger F_{st} value than neutral loci, while loci under balancing selection or purifying selection are expected to show a lower F_{st} value, which means that two wheatgrass species are undergoing more local selection (Fig. 4). Although this evolutionary process is believed to be the driving force behind sympatric speciation, we cannot predict that hexaploid populations will diversify into two species in the long term, but believe that diversification helps to increase the adaptability to the changes in altitude. Based estimates by Otto (2007), populations of hexaploid wheatgrass species may adapt to faster than populations of tetraploid wheatgrass species along altitudinal gradients because mutant alleles are less masked. Selection theory provides two probable mechanisms of heterozygote advantage and frequency-dependent selection by which diversifying selection works to maintain higher population genetic polymorphism of hexaploid species. Hexaploid *C. nutans* individuals may have heterozygote advantage compared to tetraploid *R. nutans* individuals. In the positive frequency-dependent selection, the fitness of a beneficial phenotype that adapts to the changes in altitude increases as it becomes more common, however in negative frequency-dependent selection, the fitness

of a recessive phenotype increases as it becomes less common. This finding is another proof that polyploidy wheatgrass species were not an evolutionary dead end (Otto and Whitton 2000), because higher level of polyploidy increases the ratio of marker allele associated with the altitude and the adaptation to the high-cold environment like the Qinghai-Tibetan Plateau and species richness in wild community. Once established, the long-term fate of a polyploidy lineage depends on its ability to adapt. One benefit of a higher ploidy level is that it increases the number of gene copies that can harbor a new beneficial mutation. On the other hand, as Stebbins (1971) argued, polyploidy “dilutes the effects of new mutations” because of masking by nonmutant alleles.

Selection on the genetic variation and structure of wheatgrass populations by altitude and soil conditions

Population structure and history have similar effects on the genetic diversity at all neutral loci. However, some marker loci may also have been strongly influenced by natural selection. If we could identify those loci that have responded to selection during the divergence of populations, then we may obtain better estimates of the parameters of population history by excluding these loci. In this study as well as a close relationship to altitude, the changes in the intra-population neutral genetic diversity has a relationship to the soil factors at the different altitudes (such as the available nitrogen, potassium, calcium and magnesium levels), which suggests that the altitude, soil nutrients and climate factors might play an important role in maintaining the neutral genetic diversity of wheatgrass species. Tetraploid *R. nutans* showed more response to selection from soil conditions than the hexaploid species *C. nutans*. This effect is confirmed by a previous study on the genetic diversity of *Betula luminifera* and its association with ecological factors (Xie et al. 2009).

We detected no correlation between the inter-population genetic distances and the altitude ranges in two wheatgrass species using the Mantel test, suggesting that there is no isolation due to distance between populations along altitudes, latitudes and longitudes. However, previous studies many *C. nutans* populations significant correlations between the geographical origins, altitude, and genetic distances were

observed (Chen et al. 2009; Yan et al. 2007). Differences in the sampling strategies can lead to conflicting conclusions regarding the relationship between the genetic distances and the geographical origins of the samples. Self-fertilization and limited seed dispersal can significantly influence the genetic diversity and differentiation over widely dispersed (>60 km) *Elymus* populations (Bockelmann et al. 2003). To explore the vertical changes in population genetics, rather than the horizontal changes, sampled *C. nutans* and *R. nutans* populations from a large a range of altitudes (1,200 m) within a restricted range of latitudes (<50 km horizontally). No genetic isolation by distance between populations along the altitude range must be scientifically demonstrated by notable gene flow among populations. In a previous investigations, we detected a significant gene flow from medium-altitude wheatgrass populations to low- and high-altitude wheatgrass populations (Yan et al. 2010), which can have a large influence on the linear relationship between the populations' genetic distances and altitude. Some of the above reasons are the basis of the complex genetic differentiations of the two wheatgrass species along altitudinal gradients detected in this study. Apart from the local selection above, no spatial isolation in this study and excess flow among some populations (Yan et al. 2010) have formed the complex genetic structure of two wheatgrass species.

Our findings showed a higher genetic variability in hexaploid *C. nutans* than tetraploid *R. nutans*, which is in agreement with our original hypothesis. Our study demonstrates a considerably correlation between the intra-population genetic diversity and the altitudinal distribution, suggesting that the two wheatgrass species have been under genetic selection by various environments along their altitude gradients. Tetraploid *R. nutans* has more susceptibility to undergo a stronger selection from soil condition and lower population genetic variation than hexaploid *C. nutans* population. Population-level genetic studies of polyploid plants have indicated that polyploidization should no longer be viewed as a rare event producing a polyploid species of unique origin and uniform genotype (Jeffrey 2007). Instead, polyploid species can maintain high levels of segregating genetic variation through the incorporation of genetic diversity from multiple populations of their diploid progenitors. From the above discussion on the allele share, PCoA, simulation of selection lcoi, relationship between genetic indexes

and environment along with altitude, higher adaptation of hexaploid *C. nutans* with **St**, **Y** and **H** genome than its concurrent tetraploid *R. nutans* without **H** genome is attributed to the population genetic evidence along the altitudinal gradients on the Qinghai-Tibetan Plateau found in this study.

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