Plant Ecol (2015) 216:925–937
DOI 10.1007/s11258-015-0479-3

Genetic diversity and drivers of genetic differentiation of *Reaumuria soongorica* of the Inner Mongolia plateau in China

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Received: 7 October 2014 / Accepted: 30 April 2015 / Published online: 15 May 2015

Abstract We quantified genetic diversity and gene flow among eight populations of *Reaumuria soongorica* in Inner Mongolia, China. Our results showed that genetic differentiation of *R. soongorica* across the Inner Mongolian plateau is primarily clinal in nature and is driven primarily by differential landscape resistance across areas with changing patterns of seasonal precipitation, perhaps as a result of differential timing of reproductive phenology along precipitation gradients. Finding that seasonal patterns of precipitation, and not temperature, drive population connectivity and gene flow may have important implications for predicting the effects of climate change on this keystone foundation species and devising effective strategies to utilize it in restoration efforts to ameliorate ongoing desertification in the region. Genetic diversity was highest in the western part of the sampled population, perhaps indicating that this region has historically harbored the highest effective population size of the species or may have served as the source of recent range expansion to other parts of the sampled range which exhibited lower genetic diversity. Understanding the ecological drivers of these relationships might be critical to resolving the causes of the geographical pattern of diversity, and could be important in understanding the ecology of the species sufficiently to anticipate climate change effects and effectively implement management strategies to restore the species and combat desertification.

Keywords *Reaumuria soongorica* · Reciprocal causal modeling · Landscape genetics · Climate · STRUCTURE analysis

Communicated by Siegy Krauss.

Electronic supplementary material The online version of this article (doi:10.1007/s11258-015-0479-3) contains supplementary material, which is available to authorized users.

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Introduction

Spatially heterogeneous natural selection and genetic drift can lead to population divergence even in the presence of gene flow (Coyne and Orr 2004; McKinnon et al. 2004). *Reaumuria soongorica* in the Mongolian Plateau, China, provides an excellent opportunity to investigate patterns of genetic

differentiation of peripatric populations along steep climatic gradients given that the species spans multiple climatic zones and vegetation types, across a broad range of moisture, temperature and soils, and exhibits extensive adaptability and a broad ecological niche. *R. soongorica* first occurs in the fossil record in the Cretaceous and Paleocene (Hou 1983). In the early stage of the Tertiary, there was a long arid period, during which the *R. soongorica* community developed (Yong 1990; Zhang 2005). In the desert areas of central Asia, the *R. soongorica* formation is one of the most important community types, and is the zonal desert community that is most widespread and covers the largest area in the Mongolian plateau. *R.* soongorica is widely distributed across a broad range of vegetation zones, spanning from typical desert, steppe desert, desert steppe, and typical steppe from west to east in the Mongolian plateau. In a typical desert community, *R. soongorica* is found in all soil types and is a keystone foundation species for community formation. In steppe desert, it is an adventitious community, which primarily occurs on gravelly, sandy and low saline soils. In the western part of Mongolian plateau, *R. soongorica* often is the principal dominant species, while eastward its dominance declines as aridity decreases, where it is predominantly found along saline lowlands and the peripheries of salty lakes (Editorial Committee of Flora Reipublicae Popular Sinicae, Chinese Academy of Science 1990; Inner Mongolia and Ningxia Comprehensive expedition of Chinese academy of sciences 1985; Li 1990). Given this broad ecological amplitude, the *R. soongorica* formation is the most widespread, and the most ecologically and economically important zonal community type on the Inner Mongolia plateau.

Reaumuria soongorica is a xerophytic and salt-tolerant undershrub, with strong resistance to disturbances such as soil erosion (Inner Mongolia and Ningxia Comprehensive expedition of Chinese academy of sciences 1985; Liu et al. 1982; Ma 1989; Wu 1980). Indeed, it has an extremely strong ability to stablilize shifting sand, making it extremely important in reducing effects of desertification and over grazing, which have been major environmental problems in the region over the past 50 years (Li et al. 2008; Liu et al. 1982; Ma and Kong 1998). In addition, *R. soongorica* is an important forage species, and is additionally valuable given that it is a major source of salt for livestock, and grassland dominated by *R. soongorica* is recognized as the most important grazing land in the Inner Mongolian region (Inner Mongolia and Ningxia Comprehensive expedition of Chinese academy of sciences 1985; Ma 1989; Wu 1980).

Analysis of gene flow and genetic differentiation of *R. soongorica* along this climatic gradient may allow identification of the ecological and climatic drivers of recent microevolution of this species. Additionally, understanding the factors that control gene flow and genetic diversification of *R. soongorica* may help clarify the evolutionary and ecological processes behind its keystone ecological role in plant communities of the Mongolian plateau. Due to its environmental benefit and utilization value, many studies of *R. soongorica* have been conducted on its biological characteristics (Liu et al.1982), morphology (Cui 1988), anatomy (Xiao et al. 2006), physiology (Li et al. 2005a, b), genetic diversity (Zhang et al. 2008), seed germination and breeding conditions (Zeng et al. 2004), and grazing and cultivating (Wang et al. 2002a, b) all of which have contributed to a substantial knowledge of the basic biology and management of *R. soongorica*. However, knowledge of the molecular ecology of this species is still extremely limited.

There is a lack of knowledge about genetic diversity and differentiation within and among populations of *R. soongorica* at broad scales. Recently, Li et al. (2012) identified three major chloroplast haplotype clades across the species' range that may be associated with the effects of the Qinghai-Tibet Plateau and development of desert ecosystems during the last glacial age in western China. In addition, Qian et al. (2008) found reasonably high genetic diversity and strong evidence of isolation by distance across a small portion of the species range. However, to date no studies have compared multiple hypotheses about the effects of climatic gradients on genetic differentiation and gene flow of this species. Given the very high importance of the species in efforts to ameliorate desertification and its importance to livestock production, it is essential to understand the climatic controls on the species' distribution, gene flow and genetic differentiation. In particular, proactive management depends on understanding the potential effects of climate change on the occurrence, productivity and population connectivity of this species. The distribution characteristics of *R. soongorica* in the Inner Mongolia plateau provide an opportunity to investigate the relationships between genetic diversity, gene

flow and environmental gradients. In addition, because of recent climatic changes and long-term overgrazing, *R. soongorica* has declined in extent and dominance, leading to ecological decline of the plant communities that depend on it, accelerating desertification and economic loss. Therefore, research to understand the ecology and enhance the restoration of this species is of high importance.

Investigations of genetic diversity, genetic differentiation and gene flow can provide a molecular basis for understanding the biological characteristics, evolutionary history and adaptive potential of this species, as well as its sensitivity to climate change and potential strategies to enhance its restoration. Neutral nuclear DNA markers are considered to be the most suitable means for estimating genetic diversity because of their abundant polymorphism and the fact that they are independent of environmental conditions (Gupta et al. 1994; Zietkiewicz et al. 1994). ISSR (Inter-simple sequence repeat) can provide more abundant polymorphisms and more reliable and reproducible bands relative to other DNA markers (Qian et al. 2001; Weising et al. 1995), and therefore have been widely used in detecting genetic diversity and geographic population variation, especially in distinguishing genetic variation below species levels (Song et al. 2008).

In this study, we used ISSR markers to analyze the patterns of genetic diversity within and among populations of *R. soongorica*, investigate the characteristics that enable the wide distribution of the species across the Inner Mongolia plateau, and quantify the relationships between genetic diversity, genetic differentiation, and climatic and environmental gradients. Our goal is to elucidate relationships between ecological gradients and genetic structure to provide guidance for biodiversity protection in the Inner Mongolia plateau.

Materials and methods

Study area

The Mongolian Plateau is the part of the Central Asian Plateau lying between 87°40′–122°15′N and 37°46′–53°08′E, with an area of approximately 2600,000 km^2. It is bounded by the Greater Hinggan Mountains in the east, the Yin Mountains to the south, the Altai Mountains to the west, and the Sayan and Khentii mountains to the north. The plateau includes the Gobi Desert as well as extensive dry steppe regions. It has an elevation range of approximately 700–1500 m, with the lowest point in Hulunbuir and the highest point in the Altai Mountain range.

The current study was conducted on the portion of the Mongolian Plateau within the Chinese province of Inner Mongolia. Most of Inner Mongolia is a plateau averaging around 1200 m in altitude (700–1400 m range), with a topographical cline rising from the southwest to the northeast. The soils are characterized by extensive loess and sand deposits. Soil types generally change from chestnut soil, to brown soil, to gray desert soil, and gray brown desert soil from east to west. Inner Mongolia has a wide variety of regional climates, with aridity increasing markedly from east to west. The climate is characterized by a four-season, monsoon climate, with long, cold, and dry winters, short, dry spring, and warm, relatively humid summers, except in the western parts of the region where the monsoon pattern does not reach. Mean annual temperature and temperature extremes increase from east to west ($\geq$10 °C active accumulated temperature is 1300–3700 °C). Annual precipitation is 550–10 mm, and the moisture index, which is a function of both temperature and precipitation, is 1.0–0.01, both decreasing from east to west. The natural vegetation changes in a gradient from mesic grassland and steppe in the east to desert in the west, along a chronosequence of meadow steppe (subhumid), typical steppe (semi-arid), desert steppe (drought), steppe desert (drought), and typical desert (extreme drought). Grazing is the dominant economic activity (Inner Mongolia and Ningxia Comprehensive expedition of Chinese academy of sciences 1985; Wu 1980).

Plant material

We chose eight sites along the climatic gradient from east to west across the Inner Mongolia plateau at which we collected tissues from *R. soongorica*. These sites were located in four different vegetation zones including typical steppe, desert steppe, steppe desert, and typical desert, and included several sampling locations in transitional zones (Fig. 1; Table 1). Across the eight sampling sites, both the average annual precipitation and moisture index decrease gradually.

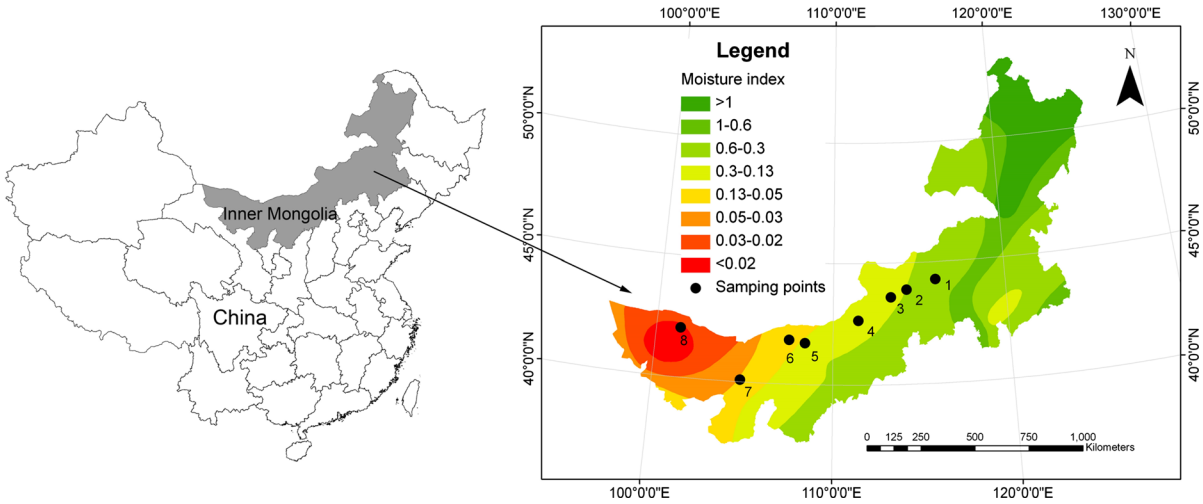

Fig. 1 Collection sites of *Reaumuria soongorica* on the Inner Mongolia plateau. Sampling sites are given in Table 1

In each sampling site, 30 individuals of *R. soongorica* were randomly selected for sampling, producing a total sample of 240 individuals. Fresh leaves collected from each selected individual were stored with silica gel in sealed plastic bags for later DNA extraction. The details on *R. soongorica* populations and the sampling sites are shown in Fig. 1 and Table 1.

DNA extraction

Total genomic DNA was extracted following the CTAB (cetyltrimethylammonium bromide) protocol (Doyle 1999). The overall quantity and quality of extracted DNA were determined in 0.8 % (w/v) agarose gels. The total DNA extracted for each sample was diluted to 20 ng/μl and stored at −20 °C.

PCR amplification

Nuclear DNA was polymerase chain reaction (PCR)-amplified using ISSR primers from the University of British Columbia (Canada). Following an initial screening of 100 random primers, 14 that gave clear reproducible fragments were selected for further analysis (Table 2). PCR was carried out in a total volume of 20 μl consisting of 10 μl of 2× Taq PCR Master Mix, 1 μl of primer, 1 μl of template DNA, and double-distilled water. All the reagents were purchased from Sangon Biotech (Shanghai, China) Co., Ltd. The amplifications were performed in a MJ Research PTC-100 thermocycler (Bio-rad, Waltham, MA, USA) programmed for an initial denaturation temperature of 94 °C for 5 min and then 40 cycles of 45 s at 94 °C, 45 s at 52 °C, 1.5 min at 72 °C, with a 5-min final extension at 72 °C. The amplification products were separated on 2.5 % (w/v) agarose gels with 0.5 g/L ethidium bromide electrophoresed in 0.5× TBE buffer (0.45 mM Tris–borate, 0.01 mM EDTA, pH 8.0) at 5 V/cm for 2 h. DNA fragments were visualized and photographed under ultraviolet light with WFH-701Type UV analyzer. Molecular weights were estimated from a 100 bp DNA ladder.

WorldClim climate layers

Bioclimatic variables derived from the monthly temperature and rainfall values are often used in ecological niche modeling (Iverson and Prasad 2002; Rehfeldt et al. 2006) or analyses of influences of climatic gradients on gene flow (Wasserman et al. 2010). We selected 18 bioclimatic variables for analysis from the WorldClim database (Hijmans et al. 2005; Table 2). WorldClim climatic layers are derived from monthly temperature and rainfall climatologies and represent biologically meaningful variables for characterizing species ranges (Nix 1986). The WorldClim database was developed using a global network of weather stations whose data were interpolated to monthly climate surfaces at 1 km spatial resolution using a thin-plate smoothing spline

Table 1 Ecological characteristics of the eight sampling sites where genetic data on *Reaumuria soongorica* were recorded on the Inner Mongolia plateau for this study

Sampling sites	Population identifier	Vegetation type	Moisture index	Type of soil	WorldClim mean annual temperature (°C)	WorldClim mean annual precipitation (mm)
1	RSI	Typical steppe	0.3–0.6	Dark chestnut soil	2.1	266
2	RSII	Typical steppe	0.3–0.6	Dark chestnut soil	2.4	224
3	RSIII	Typical steppe—desert steppe	0.13–0.3	Light chestnut soil	3.5	195
4	RSIV	Desert steppe	0.13–0.3	Brown soil	4.7	173
5	RSV	Desert steppe—steppe desert	0.13–0.3	Brown soil	4.5	197
6	RSVI	Steppe desert	0.05–0.13	Brown soil	4.4	153
7	RSVII	Steppe desert—typical desert	0.05–0.13	Gray desert soil	7.3	102
8	RSVIII	Typical desert	<0.02	Gray brown desert soil	8.9	33

Vegetation type, moisture index, and soil type as defined in Inner Mongolia and Ningxia Comprehensive expedition of Chinese academy of sciences (1985)

Vegetation type, moisture index, and type of soil were cited from Vegetation of Inner Mongolia

Table 2 List of bioclimatic variables used in analysis of gene flow

Acronym	Description
G1	Annual mean temperature
G2	Mean diurnal range (mean of monthly (max temp–min temp)
G4	Temperature seasonality (SD × 100)
G5	Max temperature of warmest month
G6	Min temperature of coldest month
G7	Temperature annual range (BIO5–BIO6)
G8	Mean temperature of wettest quarter
G9	Mean temperature of driest quarter
G10	Mean temperature of warmest quarter
G11	Mean temperature of coldest quarter
G12	Annual precipitation
G13	Precipitation of wettest month
G14	Precipitation of driest month
G15	Precipitation seasonality (coefficient of variation)
G16	Precipitation of wettest quarter
G17	Precipitation of driest quarter
G18	Precipitation of warmest quarter
G19	Precipitation of coldest quarter

algorithm with latitude, longitude, and elevation as independent variables (Hijmans et al. 2005). The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation), seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters).

Data analysis

Measures of genetic diversity

Only reproducible and unambiguous ISSR bands were scored for presence (1) or absence (0). Data were compiled into a binary data matrix using MS Excel. Population genetic parameters were analyzed using POPGENE version 1.31 (Yeh et al. 1999) to determine the percentage of polymorphic bands (PPB), Nei's (1973) gene diversity (H), Shannon's information index (I*), total genetic diversity (H_t), genetic diversity within populations (H_s), genetic diversity among populations (D_{st}), coefficient of genetic differentiation (G_{st}), Nei's (1972) genetic distance (D) between pairs of populations, and genetic identity (I). Gene flow N_m^* was calculated according to the formulae: $N_m^* = 0.5(1 - G_{st})/G_s$.

STRUCTURE analysis of genetic structure

The population genetic structure of *R. soongorica* was quantified using the Bayesian admixture procedure implemented in the program STRUCTURE (Falush et al. 2003; Pritchard et al. 2000). The program infers the genetic structure by assuming a model in which there are K (known or unknown) genetic clusters, and individual genotypes are probabilistically assigned to genetic clusters, or jointly to two or more genetic clusters if their genotypes indicate that they are admixed (Pritchard et al. 2000). Analyses were carried out with 100,000 iterations, following a burn-in period of 100,000 iterations, in order to provide insights into the genetic structure by assigning individuals to K (which ranged from 1 to 8) using only genotypic data. The most appropriate number of genetic clusters was determined based on ad hoc statistic, ΔK, which evaluates the second order rate of change in the likelihood between successive K values (Evanno et al. 2005). Three independent runs were carried out for each K value in order to assess the consistency of results.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance for the eight *R. sonngorica* populations was conducted using software Arlequin 3.11. The fixation index (FST) is widely used to estimate the genetic variation among populations. An value FST < 0.05 represents almost no genetic differentiation among populations. When $0.05 <$ FST < 0.15 there is a moderate amount of differentiation among populations. FST values between 0.15 and 0.25 represent highly differentiated among populations, and FST > 0.25 indicates very high differentiation among populations (Excoffer et al. 1992; Wright 1931).

Evaluating drivers of genetic structure

The predominant analytical approach to associate landscape patterns with gene flow processes is based on pair-wise calculation of cost distances, which are then correlated with pair-wise genetic distances among the same individuals with methods such as Mantel and partial Mantel tests (Mantel 1967; Smouse et al. 1986). There has been controversy in the literature about the appropriateness of Mantel testing in landscape genetics. Recently, Legendre and Fortin (2010) clarified this confusion, and note, that while distance-based regression approaches, such as the Mantel test, have lower power than traditional linear models and tend to underestimate the true magnitude of a relationship, partial Mantel testing remains the appropriate framework when the hypotheses are explicitly defined in terms of distance matrices, as they are in landscape genetic analyses testing effects of landscape resistance on neutral genetic differentiation.

Cushman et al. (2006) proposed a causal modeling framework to assist in model selection and increase the likelihood of identifying the true driver of genetic isolation. This approach involves identifying the most supported resistance hypothesis among a range of alternative resistance models (based on statistical significance) and then using partial Mantel tests to determine whether it meets the statistical expectations of a causal model relative to alternative models of isolation by distance or isolation by barrier. Cushman and Landguth (2010) evaluated the power of this framework and found that the method performs well in identifying the drivers of genetic differentiation in a case study complex landscape, and rejecting incorrect and correlated alternatives.

Recently, Cushman et al. (2013) found that partial Mantel tests have very low Type II error rates, but elevated Type I error rates. This leads to frequent identification of support for spurious correlations between alternative resistance hypotheses and genetic distance, independent of the true resistance model. As

a result Cushman et al. (2013) suggested several changes to the original causal modeling framework developed by Cushman et al. (2006), based on the relative support of the causal modeling diagnostic tests, rather than formal hypothesis testing. This improved method reduces the problem of false positives (Type I errors) observed by Meirmans (2012) and Amos et al. (2012).

We employ this relative support method in our analysis. Specifically, our analysis is based on computing two partial Mantel tests for each combination of alternative resistance hypotheses. For each alternative model, these two tests include: computing the partial Mantel correlation between genetic distance and the focal hypothesis, partialling out each of the other hypotheses (Test 1), and the partial Mantel correlation between genetic distance and each of the alternative hypotheses, partialling out the focal hypothesis (Test 2). The support for a particular hypothesis relative to a particular alternative is measured by the magnitude of the difference between these two tests. A large positive value for this difference indicates strong support for the first hypothesis independent of the second, and little or no support for the second independent of the first. A well supported hypothesis will have large positive values for this difference with all alternative models. In our analysis, we computed the full matrix of the difference in support for all combinations of 19 alternative resistance hypotheses (18 WorldClim climate hypotheses and Isolation by Distance) plus the two supported STRUCTURE cluster assignments ($K = 3$ and $K = 7$; e.g., Cushman et al. 2014). We evaluated which of the selected hypotheses had the most support relative to the other well supported hypotheses.

Results

Genetic polymorphism

A total of 316 bands were amplified using 14 ISSR primers with the band size ranging from 100 to 2000 bp (Table 2). On average, 22.57 bands were used per primer. All 316 loci were polymorphic (the percentage of polymorphic bands, PPB = 100 %), indicating a high level of polymorphism in genome of *R. soongorica* (Table 2).

Genetic diversity

The PPB in each *R. soongorica* population varied from 60.13 % to 75.63 %, with RSVII > RSV = RSVIII > RSIII > RSI > RSVI > RSIV > RSII. Nei's gene diversity (*H*) ranged from 0.1772 to 0.2324, with RSVII > RSVIII > RSI > RSV > RSIII > RSVI > RSII > RSIV. Shannon's information index (*I*) varied from 0.2720 to 0.3554, with RSVII > RSVIII > RSI > RSV > RSIII > RSVI > RSII > RSIV (Table 3). These three variables all show the same pattern, with genetic diversity highest for RSVII (desert region), RSVIII (desert region), and RSI (steppe region), intermediate for RSV (desert steppe—steppe desert), RSVI (steppe desert), and RSIII (typical steppe—desert steppe). The level of genetic diversity was lowest for RSII (typical steppe) and RSIV (desert steppe). These results suggest that genetic diversity does not increase or decrease monotonically with aridity. For all measures of genetic diversity assessed, we found the highest genetic diversity of the species was in the western desert habitats.

Table 3 Analysis of genetic diversity across 316 loci for eight populations using ISSR markers, with 22.57 loci per primer

Population identifier	Percentage of polymorphic loci % (PPB)	Nei's gene diversity (*H*)	Shannon's information index (*I*)
RSI	64.87	0.2096	0.3163
RSII	60.13	0.1805	0.2762
RSIII	66.46	0.2047	0.3116
RSIV	60.44	0.1772	0.2720
RSV	68.04	0.2071	0.3161
RSVI	62.03	0.1906	0.2920
RSVII	75.63	0.2324	0.3554
RSVIII	68.04	0.2110	0.3216

Genetic differentiation and gene flow

The Nei's coefficient of genetic differentiation (G_{st}) for populations of *R. soongorica* was 0.1931, indicating that 19.31 % of total variation occurred among the populations and 80.69 % occurred within populations, and gene flow (N_m^*) was 2.0899, indicating substantial genetic migration among populations. AMOVA showed that 26.23 % of total variation occurred among the populations and 73.77 % occurred within populations.

Fixation index for populations of R. soongorica was 0.26233 (>0.25) suggesting there is high genetic differentiation among populations. However, the value of gene flow (N_m^*) for the populations was $N_m = 2.0899$, indicating a moderate level of genetic exchange between populations, which may prevent genetic differentiation among populations due to genetic drift. These results suggest partial isolation of populations and varying levels of gene flow, suggesting clinal population structure.

Bayesian analyses

Cluster results of STRUCTURE revealed three or seven genetic clusters confirmed by calculation of ΔK (Fig. 2). In the $K = 7$ solution, all populations were assigned to single clusters by themselves, except for population RSV and RSVII which formed one genetic cluster (Fig. 3a). This suggests that the genetic structure of RSV and RSVII are similar, indicating relatively high gene flow among these populations

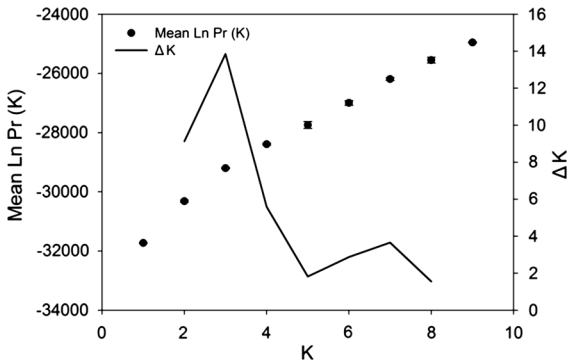

Fig. 2 Mean LnPr (K) over three runs for each K value and ΔK obtained with Bayesian analyses implemented in the software STRUCTURE

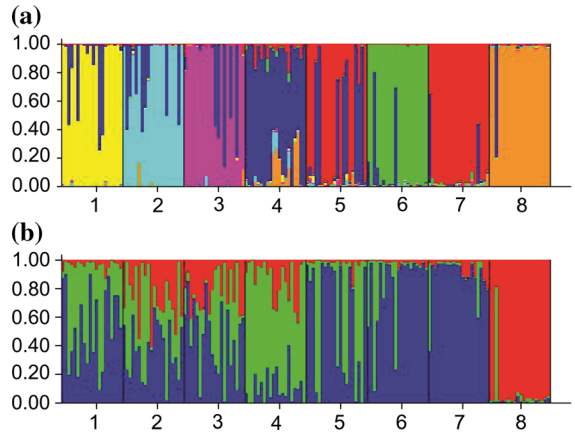

Fig. 3 Results of Bayesian analysis for 8 populations of *R. sonngorica* from STRUCTURE under admixture model for **a** $K = 7$ and **b** $K = 3$. *1* RSI, *2* RSII, *3* RSIII, *4* RSIV, *5* RSV, *6* RSVI, *7* RSVII, *8* RSVIII

which are both located in the ecological transition zone.

In the $K = 3$ solution, RSVIII individuals were assigned into a single cluster. The clustering indicated similar genetic structure of individuals from RSV, RSVI, and RSVII. The high degree of admixture indicated that there is considerable genetic exchange among the eight populations, with particularly strong gene flow among RSI, RSII, RSIII, RSIV, and RSV (Fig. 3b).

Reciprocal causal modeling with Mantel tests

Reciprocal causal modeling across 19 resistance hypotheses and two structure groupings revealed that a single hypothesis had strong support based on the matrix of difference in support between partial Mantel test 1 and partial Mantel test 2 (Fig. 4). Specifically, interseasonal variability in precipitation (G15) was well supported compared to all alternative hypotheses (positive values in columns associated with these hypotheses in Fig. 4). In contrast, none of the other 17 alternative resistance hypotheses or isolation by distance were supported, and none had any support independent of G15 (negative or zero values in the rows associated with these hypotheses in Fig. 4). There was some residual support for the two STRUCTURE cluster solutions ($K = 3$ and $K = 7$) independent of interseasonal variability in precipitation (G15), indicating some genetic structure not

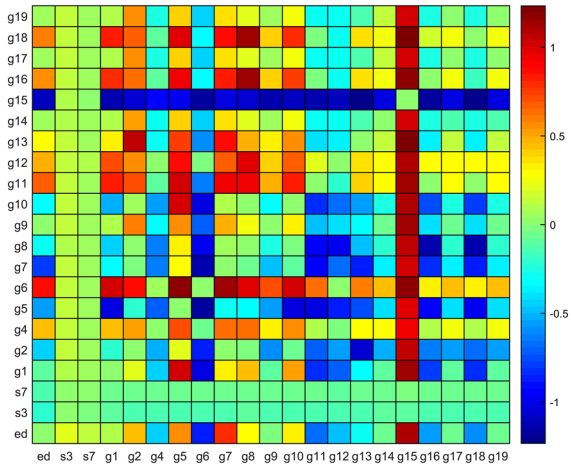

Fig. 4 Matrix of difference in support for column hypotheses relative to row hypotheses. The values in the table are the difference in partial Mantel r for test 1 (genetic distance ~ *column* hypothesis | *row* hypothesis) and test 2 (genetic distance ~ *row* hypothesis | *column* hypothesis). *Positive values* indicate support for the *column* hypothesis relative to the *row* hypothesis. The *matrix* shows full support for hypothesis g15, precipitation seasonality, and no other hypotheses are supported

accounted for by this landscape resistance model that seems to be associated with three or seven genetic clusters.

Discussion

Isolation by Climatic Gradients

Our results indicated strong genetic differentiation of *R.soongorica* across the Mongolian Plateau, and that this genetic differentiation was primarily clinal in nature, but also had some attributes independently associated with Bayesian clustering. Gene flow among populations is quite high ($N_m = 2.0899$), but considerable genetic differentiation still exists among populations (FST = 0.26233), consistent with gene flow along a cline. STRUCTURE analysis revealed weak clustering with high degree of admixture, also suggesting a clinal gradient of gene flow across the study area. Schwartz and McKelvey (2009) found that Bayesian clustering approaches, such as STRUCTURE, detect spurious clusters when sampling is spatially segregated along a genetic cline. Our sampling was spatially distributed across several hundred kilometers, with sampling "sites" representing clustered sampling along this cline. Given the Schwartz and McKelvey (2009) findings, we would expect STRUCTURE to identify clusters from this sampling regime even if the underlying genetic structure was continuous and clinal in nature. Indeed, this was one of the main motivations for our use of comprehensive analysis with reciprocal causal modeling to directly compete clustering, isolation by distance and a range of isolation by resistance hypotheses. Our analysis was able to evaluate the relative and independent support among all candidate hypotheses and confirmed both a dominant pattern of clinal variation in genetic differentiation associated with gradients of seasonal precipitation and also some weaker residual structure associated with genetic clusters. The residual support for STRUCTURE groups may reflect patterns of past population fission/fusion and expansion from refugia over long time scales (e.g., Dyer et al. 2010; Cushman 2015). For example, Li et al. (2012) studied effects of past climatic events on intraspecific divergence and range shifts in *Reamuria soongorica* using chloroplast DNA from 27 natural populations across western China. They found eight haplotypes clustered into three clades divided between eastern and western china. They surmised that the resulting pattern indicated past regional range expansion corresponding to the uplift of the Qinghai-Tibet Plateau and development of desert ecosystems during the last glacial age. Our analysis is unable to test that hypothesis, but our observation of residual structure most strongly associated with three genetic clusters could plausibly reflect the pattern of population differentiation described by Li et al. (2012). Like Cushman et al. (2014), we found both strong clinal patterns in genetic differentiation related to climatic gradients and also independent underlying discrete patterns of genetic clustering simultaneously exist across the population.

Qian et al. (2008) used ISSR markers across seven subpopulations of *Reamuria soongorica* in a relatively small area in western China. Similar to our results, Qian et al. (2008) found relatively strong genetic structure coupled with relatively high gene flow. They analyzed isolation by distance with Mantel tests and found strong correlation between genetic differentiation and geographical distance. Our results provide a next step beyond those of Qian et al. (2008) in that we directly competed 21 alternative hypotheses, including isolation by distance and STRUCTURE

groupings, and found that isolation by distance was not independently supported and that the dominant drivers of gene flow are related to climatic gradients.

Interestingly, we found that variation in precipitation is a much stronger predictor of genetic differentiation among populations of *R. soongorica* than is variation in temperature. This suggests that gradients of changing precipitation may act as resistant factors limiting gene flow, and provide stronger prediction of genetic differentiation than isolation by distance, and that variation in temperature has little relation to gene flow. A very similar result was found for *Populus fremontii* by Cushman et al. (2014). They found no support for genetic differentiation based on differences between climatic zones or point climatic conditions at the sites of populations, but strong support for increased genetic differentiation as a function of cumulative difference in winter and spring precipitation between populations. This suggests that seasonal differences in precipitation result in reduced gene flow, plausibly due to the effects on flowering phenology. We posit that the same process may be occurring in *R. soongorica* across the Mongolian Plateau, with differences in seasonal precipitation driving differences in flowering phenology, leading to cumulative isolation by climatic gradients.

Yang et al. (2013) found similar relationships between gene flow and variation in seasonal precipitation for a clade of *Caragana* species on the Ordos Plateau, which is a subregion of the current study. That study found that gene flow within and among *Caragana* populations was driven primarily by winter precipitation and precipitation seasonality, with no independent relationship with other climate gradients or isolation by distance. The results in this study are similar, except that we found no support for a relationship between gradients of winter precipitation and gene flow, and only found that variation in seasonal precipitation across space acted as a resistant factor attenuating gene flow. The general similarity of the results suggests that seasonal variation of precipitation may act as a structuring factor limiting gene flow for a range of plant taxa across the Mongolian Plateau, and that other climate factors seem to play a lesser role. This knowledge could be of great value in projecting potential effects of climate change on population migration and gene flow of *R. soongorica* and potentially other plant species as well.

The relationship between seasonal variation in precipitation and gene flow observed in this study and in the Yang et al. (2013) study may be driven by the effects of climate on phenology. Climate conditions, especially precipitation and temperature, will affect growth and phenology. For flowering plants in the temperate zone, if it is warm with ample rain in the spring, plants will leave dormancy and enter active growth earlier than in cold and dry locations. If precipitation is low in summer and autumn, the period of active growth will terminate early and plants will enter dormancy earlier than in areas with higher summer and autumn precipitation. Likewise, precipitation in autumn and winter can significantly affect the timing of the next year's bud-burst and flowering. Specifically, if precipitation of the preceding autumn and winter was high, bud-burst and flowering will both occur earlier in the year than average (Bai et al. 2010; Guo et al. 2012; Song et al. 2012a, b).

Across the Mongolian Plateau, there is very little precipitation in spring. Therefore, differences in winter and summer precipitation likely have very strong effects on the ecophysiological factors controlling flowering and other reproductive processes. Our results are consistent with the hypothesis that differences in flowering phenology along gradients of changing precipitation seasonality may influence pollination and seed diffusion, and drive differential patterns of gene flow. In addition, the differential climatic conditions across the precipitation gradient may impose marked local directional selection on the local populations (Yang et al. 2013). These climatic differences likely reduce fitness of locally maladapted individuals, resulting in population divergence and maintenance of reproductive isolation (de León et al. 2010; Gavrilets 2000; Gavrilets and Vose 2007; Niemiller et al. 2008; Nosil 2008).

Patterns of genetic diversity

Our genetic analysis revealed that *R. soongorica* populations across the Inner Mongolian plateau exhibit abundant genetic diversity. The amount of genetic diversity in plant populations reflects the result of evolutionary actions, including accumulation of adaptive genetic changes to environmental gradients. High genetic diversity is associated with greater evolutionary potential and the ability for breeding

and genetic improvement (Beebee and Rowe 2003; Chai et al. 2010; Jiang 2004). Such high genetic diversity is not unexpected given that *R. soongorica* is widely distributed across typical desert, steppe desert, desert steppe, and typical steppe in the region.

For all measures of genetic diversity assessed, we found the highest genetic diversity of the species was in the western desert habitats. This was expected, as it is generally to be expected that genetic diversity will be highest in the main distribution area of a species' range where effective population size is highest, and decrease toward the periphery or in areas where the range is expanding or populations are fluctuating widely. The lower genetic diversity in the middle and eastern parts of the range suggest perhaps that populations of *R. soongorica* may have fluctuated or that local migration may have occurred that has reduced genetic diversity in these areas, and that conversely, the populations in the far western part of the study area may have had a longer stable period with relatively high effective population size. Understanding the causes of this expected pattern of genetic diversity could be important to piecing together the ecological history of the species in the Mongolian Plateau and anticipating the effects of climate change, and effectively using the species to mitigate desertification.

Conclusion

Our modeling results do not support isolation by distance as a significant factor in determining genetic differentiation in *R. soongorica* after accounting for landscape resistance due to climate gradients. STRUCTURE clustering identifies grouping of genetically similar populations without any a priori hypotheses of driving factors. Given they lack any a priori basis these clusters are observations of differentiation and not explanations. We treated isolation by STRUCTURE clustering groupings as null models in this analysis, and our finding that there is residual independent support for them after accounting for isolation by distance and isolation by resistances suggests that these patterns are not merely spurious results of clustered sampling along clinal population gradients (Schwartz and McKelvey 2009; Cushman and Landguth 2010). Genetic structure of *R. soongorica* population is primarily related to gradients of increasing isolation by climatic differences (landscape resistance), but this model leaves a portion of variance in spatial genetic structure unexplained. This residual variation is related to other factors not evaluated in the model, but is correlated with the STRUCTURE groups, and may reflect patterns of past population fission/fusion and expansion from refugia over long time scales (e.g., Dyer et al. 2010), as described for this species in Li et al. (2012).

We demonstrated that genetic differentiation of *R. soongorica* across the Mongolian Plateau is clinal in nature and is driven primarily by differential landscape resistance across areas with changing patterns of seasonal precipitation. Changing seasonality of precipitation probably limits gene flow by affecting phenology of flowering events that lead to increased prezygotic isolation among populations. Finding that seasonal patterns of precipitation, and not temperature, drive population connectivity, and gene flow may have important implications for predicting the effects of climate change on this keystone foundation species and devising effective strategies to utilize it in restoration efforts to ameliorate ongoing desertification in the region. For example, further research would be warranted to determine if the climatic differentiation we observe is associated with any adaptive differences among populations. Such adaptive differences would be essential to guide selection of seed sources for restoration plantings that are adapted to the climate of the target environment. In addition, understanding the climatic controls on gene flow across the Mongolian Plateau could help guide conservation and land management by clarifying how naturally connected populations are and where gene flow is most attenuated. This, in turn, could guide restoration efforts to maintain gene flow by placing stepping stone populations across areas of steep climatic change to enable gene flow through these highly resistant zones.

We found that genetic diversity was highest in the western part of the sampled population, perhaps indicating that this region has historically harbored the highest effective population size of the species or may have served as the source of recent range expansion to other parts of the sampled range which exhibited lower genetic diversity. Understanding the ecological drivers of these relationships might be critical to resolving the causes of the geographical pattern of diversity, and could be important in understanding the ecology of the species sufficiently

to anticipate climate change effects and effectively implement management strategies to restore the species and combat desertification.

Acknowledgments This work was supported by The State Key Basic Research Development Programme of China (Grant No. 2012CB722201),science and technology research projects of Inner Mongolia Education Department of China (NJ10002), The Natural Science Foundation of Inner Mongolia Autonomous Region of China (2014MS0350), and US Forest Service Rocky Mountain Research Station.

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