Landscape Ecol
DOI 10.1007/s10980-013-9913-x

RESEARCH ARTICLE

Effects of climatic gradients on genetic differentiation of *Caragana* on the Ordos Plateau, China

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Received: 10 December 2012 / Accepted: 24 June 2013

Abstract The genus *Caragana* (Fabr.) in the Ordos Plateau of Inner Mongolia, China, provides a strong opportunity to investigate patterns of genetic differentiation along steep climatic gradients, and to identify the environmental factors most likely to be responsible for driving the radiation. This study used a factorial, multi-model approach to evaluate alternative hypotheses and identify the combination of environmental factors that appear to drive genetic divergence of *Caragana* in the Ordos Plateau. We had three specific hypotheses. First, we expected that gradients of changing climate would act as resistant factors limiting gene flow, and would provide stronger prediction of genetic differentiation than isolation by distance. Second, we expected that variation in precipitation would be a stronger predictor of genetic differentiation among populations than variation in temperature. Third, we expected that the pattern of phylogenetic differences, in terms of derived versus ancestral states of rachis and leaf shape, would be highly correlated with these gradients of changing precipitation, reflecting adaptive radiation along gradients of changing precipitation driven by reduced gene flow and differential patterns of directional selection. As we expected, variation in precipitation was a much stronger predictor of genetic differentiation than were other climatic variables or isolation by distance. The pattern of phylogenetic differentiation among *Caragana* species is also closely associated with gradients of changing patterns of precipitation, suggesting that differential precipitation plays a major role in driving the genetic differentiation and adaptive radiation of the *Caragana* genus in the region of the Ordos Plateau.

Keywords *Caragana* · Landscape genetics · Adaptive radiation · Gene flow · Climate gradients

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Introduction

Spatially heterogeneous natural selection and genetic drift can lead to population divergence and speciation, even in the presence of gene flow (Coyne and Orr 2004; McKinnon et al. 2004). Simulation studies have suggested that parapatric speciation is possible due solely to limited dispersal distances and accumulation of genetic incompatibilities (e.g. Gavrilets et al. 2000; Hoelzer et al. 2008). In addition, adaptation to local environments can be a major driver of speciation (Wright 1932; McKinnon et al. 2004). Such ecological speciation has been shown to be plausible even in the

Published online: 20 August 2013

presence of gene flow (Niemiller et al. 2008; Nosil 2008). One of the major drivers of ecological speciation is differential timing of reproductive events (Feder et al. 1993; Yamamoto and Sota 2009).

The genus *Caragana* (Fabr.) in the Ordos Plateau of Inner Mongolia, China, provides an unusual opportunity to investigate patterns of adaptive radiation and gene flow along steep climatic gradients, and to identify the environmental factors most likely to be responsible for driving the radiation. *Cargana* belongs to the family *Leguminosae*, and consists of about 100 species, distributed mainly in arid and semi-arid regions of Asia and Europe. There are more than 60 species of *Caragana* in China (Editorial Committee of Flora Reipublicae Popular Sinicae, Chinese Academy of Science 1993). The *Caragana* populations in north-central China provide a unique opportunity to explore drivers of genetic differentiation and adaptive radiation for several reasons. While the biological species concept is based on reproductive incompatibility, the species in this study are not reproductively incompatible, and readily hybridize when in proximity. Thus, it is appropriate to evaluate neutral gene flow among them, which provides a novel opportunity to evaluate the relative effect of geographical and environmental isolation, and determine how well taxonomic/morphological divergence is congruent with genetic isolation by climatic gradients. If taxonomic and morphological divergence are highly consistent with observed patterns of genetic differentiation, and if these are explainable by particular environmental gradients, this would provide ecological explanations for the radiation or maintenance of differentiation of *Caragana* populations.

The original mesophile species of *Caragana* originated in East Asia and spread across the Asian continent by $\sim$30 million years ago, facilitated by uniformly mesic Teriary climatic conditions (Komarov 1908, 1945; Sanczir 1979; Zhou 1996; Zhang 1998). At the end of the Miocene (6 million years ago) the ancient Mediterranean Sea retreated westward as the Qinghai-Tibet Plateau uplifted strongly, driven by the impact of the Indian subcontinent with Eurasia. As the Qinghai-Tibet Plateau was uplifted, the climate gradually became drier in interior Asia (Manabe and Terpstra 1974; Ye and Gao 1979; Manabe and Broccoli 1990; Kutzbach et al. 1993; Zhou 1996; Liu et al. 2001). This dramatic climatic change likely directly affected gene flow and directional selection in the *Caragana* taxa. Steep climatic gradients likely limited gene flow and imposed strong selective forces on local *Caragana* populations. The degree of aridity increases from east to west in interior Asia, with drought adapted *Caragana* species predominating in the arid areas of central Asia, while mesic adapted species are most common in the moist areas of East Asia.

There is a consensus among taxonomists that the ancestral *Caragana* taxa had pinnately compound leaves, while pseudo-palmately compound leaves are present in some modern *Caragana* species. Similarly, the ancestral taxa is believed to have had deciduous rachis, while persistent and hardening rachis is a derived feature found in some modern species (Dormer 1945; Gorbunova 1984; Zhang 1997).The *Caragana* species presently occurring in the Ordos Plateau include species with both pinnately compound leaves and pseudo-palmately compound leaves, and species with deciduous rachis and persistent and hardening rachis. The pattern of occurrence of these traits among *Caragana* populations, therefore, can serve as a means to estimate phylogenetic distance among species based on shared plesiomorphic and apomorphic traits (Wiley 1991), and a means to evaluate relationships between the distribution of these traits among populations and climatic gradients on the Ordos Plateau.

Previous studies of *Caragana* evolutionary differentiation and speciation focused on the geographical distribution, morphological characteristics, cytology, and pollen morphology of different *Caragana* populations (Yang et al. 1990a, b; Zhang et al. 1996; Chang and Zhang 1997; Zhang 1998; Ma et al. 2003; Yang et al. 2012). However, the influence of climatic factors on gene flow and genetic differentiation of the *Caragana* populations has not been investigated. The goal of this paper is to evaluate the relationships between multiple climatic gradients and genetic differentiation among the *Caragana* populations found on the Ordos Plateau. We employ an improved version of the causal modeling approach (Cushman et al. 2006; Cushman et al. 2013) to evaluate multiple climatic gradients, isolation by distance, and phylogenetic distance as potential drivers of genetic differentiation among Ordos Plateau *Caragana* species.

We used partial Mantel tests to evaluate alternative relationships between bioclimatic variables and gene flow in genus *Caragana* populations in the Ordos

Plateau. Our primary goal was to determine the relative influences of isolation by geographical distance and climatic gradients in explaining genetic differentiation among *Caragana* populations. We were particularly interested in evaluating the congruence of phylogenetic divergence (as measured by several morphological traits) with observed patterns of genetic differentiation, and determining if this divergence was predictable based on gradients of climatic difference across the study area. We had three specific hypotheses. First, we expected that gradients of changing climate would act as resistant factors limiting gene flow, and would provide stronger prediction of genetic differentiation than isolation by distance. Second, we expected that variation in precipitation would be a stronger predictor of genetic differentiation among populations than variation in temperature. This is because there is relatively little altitudinal change across the Ordos Plateau, resulting in relatively constant mean temperatures, while there is a strong gradient of increasing aridity from the mesic eastern part of the plateau to the extremely arid western edge. Thus, we expected that differences in temperature would not limit gene flow among these populations, while differences in precipitation would. Third, we expected that the pattern of phylogenetic differences, in terms of derived versus ancestral states of rachis and leaf shape, would be highly correlated with these gradients of changing precipitation, reflecting adaptive radiation along gradients of changing precipitation driven by reduced gene flow and differential patterns of directional selection. This third hypothesis is motivated by the expectation that observed phylogenetic and morphological differentiation should reflect a history of divergence driven by a combination of reduced gene flow and directional selection to local climatic conditions. Thus we would expect phylogenetic and morphological differentiation to be congruent with observed patterns of genetic differentiation, and that these would be predictable based on climatic differences.

Methods

Study area

The study was conducted in the Ordos Plateau, located at 37°38'–40°52′N, 106°42' –111°28′E in the Chinese Province of Inner Mongolia. The plateau's area is approximately 130,000 square kilometers in extent. The geological history of the Ordos region is complex and likely provides critical information about the drivers of evolution in the *Caragana* genus. In the late Miocene and early Pliocene, the Ordos Plateau region transitioned from a basin to a plateau, driven by the uplifting forces generated by the impact of the Indian Subcontinent with Eurasia. The climate change caused by the uplift was mainly characterized by increasing aridity, driven by the orographic effects of the Qinghai-Tibet Plateau blocking the East Asia monsoon circulation and Siberia–Mongolia high pressure influence (Dong et al. 1983; Li 1990; Chen et al. 1999; Grubov 1999; Yue et al. 2007).

The Ordos Plateau spans a climatic gradient from areas experiencing weak monsoon (eastern portion of the plateau), semi-arid (central portion), and arid (western portion). Site-level mean annual precipitation ranges from 160 to 450 mm, decreasing from east to west. Mean annual temperatures range from 5.0 to 9.2 °C and ≥10 °C active accumulated temperature rages from 2,400 to 3,650 °C (Li 1990). As the climate becomes drier as one moves from east to west, the vegetation communities change in sequence from meadow steppe, to typical steppe, to desert steppe and finally steppe desert at the western edge of the Ordos Plateau.

Shrubs are the most conspicuous life form of plants on the Ordos Plateau (Li 1990; Li 1997). There are some 123 shrub species found in the study area, including 35 semishrub species, which belong to 30 families and 58 genera. The high shrub diversity of the Ordos Plateau is a significant characteristic not only of this arid and semi-arid region, but also of the temperate steppe zone of China in general. Moreover, the Ordos Plateau is regarded as the source of shrub diversity of the entire Chinese temperate steppe. Consequently, the region is a crucial area for shrub diversity conservation (Li 2000).

Relative to other genera, the genus of *Caragana* is the most abundant shrub taxa on the Ordos Plateau (Chen et al. 1999). It includes *Caragana purdomii* Rehd., *C. opulens* Kom., *C. intermedia* kuang et H.C. Fu, *C. korshinskii* Kom., *C. stenophylla* Pojark., *C. roborovskyi* Kom., *C. tibetica* Kom. and *C. brachypoda* Pojark. These species grow in the typical steppe, desert steppe, and steppe desert along the east–west gradient of the plateau and form various vegetation patterns, such as mixed communities, and

interleaved or fragmented (vicariance) distribution patterns (Ma 1989; Li 1990). *Caragana* plants are of high ecological and economic importance in this ecologically fragile region. They play an important role in maintaining biodiversity, reducing wind, and ameliorating soil erosion resulting from livestock heavy grazing (Wu 1980; Ma 1989; Li 1990; Li 2000).

Genetic sampling

We chose 11 sites from east to west across the Ordos Plateau in the province of Inner Mongolia, China (Fig. 1). The sites were selected to span the gradient of moisture coefficient (Li 1990) that exists on the Mongolian Plateau. This gradient runs from moister conditions to the east to drier, desert conditions to the west, and covers three different vegetation zones including typical steppe ($K > 0.23$), desert steppe ($0.13 < K < 0.23$) and steppe desert ($K < 0.13$; Li 1990; Fig. 1; Table 1). Each sampling site consisted of a 50 m × 50 m quadrat in which we randomly sampled 20 individuals from each *Caragana* species present. A total of 20 *C. opulens*, 20 *C. purdomii*, 60 *C. stenophylla*, 40 *C. intermedia*, 40 *C. korshinskii*, 20 *C. roborovskyi*, 40 *C. tibetica*, and 20 *C. brachypoda* individuals were sampled. Fresh leaves collected from each selected individual were stored with silica gel in sealed plastic bags for later DNA extraction. The details on *Caragana* populations and the sampling sites are shown in Table 1.

Genetic analysis

Total genomic DNA was extracted following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle 1999). The overall quantity and quality of extracted DNA were determined in 0.7 % (w/v) agarose gels. The total DNA extracted for each sample was diluted to 20 ng/μl and stored at −20 °C. DNA was amplified with polymerase chain reaction (PCR) using ISSR primers from the University of British Columbia (Canada). Following an initial screening of 20 random primers, 14 that gave clear reproducible fragments were selected for further analysis (Table 2). PCR was carried out in a total volume of 25 μl consisting of 1× PCR buffer [100 mM KCl, 80 mM $(NH4)_2$ SO_4, 100 mM Tris–HCl, pH 9.0], 0.05 mM $MgCl_2$, 0.005 mM dNTP, 1.5 U of Taq polymerase, 1 μl of primer, 20 ng 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 1.5 min and then 45 cycles of 45 s at 94 °C, 1 min at 54 °C and 1.5 min at 72 °C, with a 5-min final extension at 72 °C. The amplification products were separated on 1.5 % (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

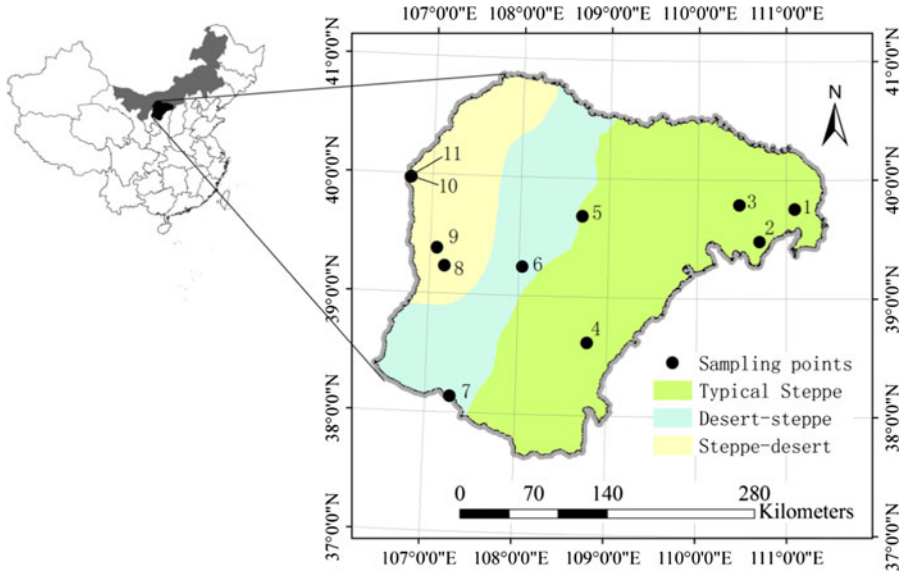

Fig. 1 Collection sites of genus *Caragana* species on the Ordos Plateau. Sampling sites are given in Table 1

Table 1 Details of *Caragana* species and sampling sites on the Ordos Plateau

Sampling sites	Species	Population identifier	Life form	Vegetation type	Moisture coefficient[a]
1	*C. opulens* Kom.	CO	Meso-xeric shrub	Typical steppe	>0.4
2	*C. purdomii* Rehd.	CP	Xeric-meso shrub	Typical steppe	0.4
3	*C. stenophylla* Pojark.	CSI	Strong xeric undershrub	Typical steppe	0.3–0.4
4	*C. intermedia* kuang et H.C.Fu	CII	Xeric shrub	Typical steppe	0.3–0.4
5	*C. korshinskii* Kom.	CKI	Xeric shrub	Typical steppe	0.23–0.3
6	*C. intermedia* kuang et H.C.Fu	CIII	Xeric shrub	Desert steppe	0.2–0.23
6	*C. stenophylla* Pojark.	CSII	Strong xeric undershrub	Desert steppe	0.2–0.23
7	*C. roborovskyi* Kom.	CR	Strong xeric undershrub	Desert steppe	0.2–0.23
7	*C. tibetica* Kom.	CTI	Strong xeric undershrub	Desert steppe	0.2–0.23
8	*C. tibetica* Kom.	CTII	Strong xeric undershrub	Steppe desert	<0.13
9	*C. stenophylla* Pojark.	CSIII	Strong xeric undershrub	Steppe desert	<0.13
10	*C. brachypoda* Pojark.	CB	Strong xeric undershrub	Steppe desert	<0.13
11	*C. korshinskii* Kom.	CKII	Xeric shrub	Steppe desert	<0.13

[a] Moisture coefficient uses the formula of H. H. Evenoff's moisture coefficient (Li 1990)

Table 2 Sequence of ISSR primers and amplification results

Primer	Sequence of primer (5′ → 3′)	Number of bands	Number of polymorphic bands	Percentage of polymorphic bands % (PPB)
AW64746	ACACACACACACACACT	16	16	100
AW64747	ACACACACACACACACAG	20	20	100
AW64749	TGTGTGTGTGTGTGTGC	14	14	100
AW64750	CTCCTCCTCCTCCTCCTC	32	32	100
AW64751	TATTATTATTATTATTAT	38	38	100
AW77934	AGAGAGAGAGAGAGAGC	32	32	100
AW77935	AGAGAGAGAGAGAGAGG	27	27	100
AW77936	GAGAGAGAGAGAGAGAC	27	27	100
AW77937	CACACACACACACACAG	10	10	100
AW77938	GAGAGAGAGAGAGAGAA	17	17	100
AW77939	AGAGAGAGAGAGAGAGTC	34	34	100
AW77940	GAGAGAGAGAGAGAGAGG	23	23	100
AW77941	GAGAGAGAGAGAGAGAAT	34	34	100
AW77943	GGAGAGGAGAGGAGA	36	36	100
Total		360	360	100

visualized and photographed under ultraviolet light with a WFH-701 Type UV analyzer. Molecular weights were estimated from a 100-bp DNA ladder.

The ISSR bands were scored as presence (1) and absence (0). Table 3 shows the amplification results of *Caragana* populations by ISSR primers. Population genetic parameters were analyzed using POPGENE version 1.31 (Yeh et al. 1999) to determine Nei's (1972) genetic distance (D) between pairs of populations (Table 4).

Resistance hypotheses

Bioclimatic variables derived from the monthly temperature and rainfall values are often used in ecological niche modeling (Iverson and Prasad 2002;

Table 3 Amplification results of *Caragana* populations by ISSR primers

Population identifier	Number of bands	Number of polymorphic bands	Percentage of polymorphic bands % (PPB)	Bands/primer
CII	191	165	86.39	13.64
CIII	208	197	94.71	14.85
Mean	199.5	181	90.55	28.49
CKI	160	139	86.44	11.43
CKII	199	182	91.46	14.21
Mean	179.5	160.5	88.95	12.82
CSI	203	202	99.51	14.50
CSII	183	167	91.26	13.17
CSIII	199	188	94.47	14.21
Mean	292.5	185.67	95.08	13.96
CTI	185	176	95.14	13.21
CTII	173	154	89.02	12.36
Mean	179	165	92.08	12.79
CP	155	128	82.58	11.07
CO	148	113	76.35	10.57
CR	178	154	86.52	12.71
CB	126	54	42.86	9.00

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 5). WorldClim climatic layers are derived from monthly temperature and rainfall climatologies and represent biologically meaningful variables for characterizing species ranges (Nix 1986). The WorldClim data base 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 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).

Phylogenetic distance

We calculated an index of phylogenetic distance between all pairs of *Caragana* populations based on two morphological traits that indicate phylogenetic grouping. First, the genus *Caragana* can be divided into two groups of species according to their leaf morphology. The plesiomorphic group has pinnately compound leaves, while the apomorphic group has pseudo-palmately compound leaves. *Caragana* species, *C. intermedia*, *C. korshinskii*, *C. tibetica*, *C. roborovskyi* and *C. purdomii* are typical species of the pinnately

Table 4 Genetic distance (*D*) between pairs of populations of *Caragana* Fabr. revealed by ISSR markers

Population identifier	CO	CP	CSI	CII	CKI	CIII	CSII	CR	CTI	CTII	CSIII	CB	CKII
CO	****												
CP	0.2267	****											
CSI	0.1788	0.1296	****										
CII	0.2062	0.1628	0.1346	****									
CKI	0.2048	0.1447	0.1032	0.1223	****								
CIII	0.1688	0.1124	0.0889	0.1169	0.0774	****							
CSII	0.1974	0.1656	0.0665	0.1528	0.1477	0.125	****						
CR	0.1998	0.1722	0.1257	0.186	0.1509	0.1361	0.1707	****					
CTII	0.1638	0.1741	0.0838	0.1512	0.1224	0.1049	0.1127	0.1514	****				
CTII	0.1951	0.1693	0.1077	0.1694	0.1345	0.1399	0.1471	0.1654	0.0906	****			
CSIII	0.1927	0.1561	0.0527	0.159	0.1276	0.1183	0.0774	0.1499	0.1073	0.1343	****		
CB	0.3198	0.3454	0.2412	0.3066	0.2871	0.2846	0.2707	0.317	0.29	0.2905	0.2735	****	
CKII	0.2002	0.1635	0.1156	0.1237	0.0813	0.1044	0.1559	0.1714	0.1203	0.1557	0.144	0.3114	****

* indicates the diagonal in the symmetrical n×n table. It is used only to facilitate interpretation by demarking the self-self comparisons

Table 5 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 (standard deviation * 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

compound leaf group, while *C. opulens, C. stenophylla* and *C. brachypoda* belong to pseudo-palmately compound leaf group. Second, species are grouped according to their leaf rachis morphology. The plesiomorphic group has rachis that are shed, and includes *C. purdomii*, *C. korshinskii*, *C. intermedia*, while the apomorphic group has persistent rachis, and consists of *C. roborovskyi*, *C. tibetica*, *C. stenophylla*, *C. brachypoda* and *C. Opulens*. (Yang et al. 1990a, b; Editorial Committee of Flora Reipublicae Popular Sinicae, Chinese Academy of Science 1993; Zhou et al. 1994; Zhang et al. 2002; Zhou et al. 2005).

We developed an index of phylogenetic distance based on the plesiomorphic vs apomorphic traits of each species. A species was given a phylogenetic distance of 0 from itself. A pair of species was given a phylogenetic distance of 1 if they both shared the same characteristics for both traits. A pair of species was given a phylogenetic distance of 2 if they shared one trait but differed on the second. Finally, a phylogenetic distance of 3 was assigned if a pair of species differed in both leaf morphology and rachis persistence (Table 6).

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

Table 6 Phylogenetic distance between pairs of populations of *Caragana* Fabr.

Population identifier	CO	CP	CSI	CII	CKI	CIII	CSII	CR	CTI	CTII	CSIII	CB	CKII
CO	0	3	1	3	3	3	1	2	2	2	1	1	3
CP	3	0	3	1	1	1	*3*	2	2	2	3	3	1
CSI	1	3	0	3	3	3	0	2	2	2	0	1	3
CII	3	1	3	0	1	0	3	2	2	2	3	3	1
CKI	3	1	3	1	0	1	3	2	2	2	3	3	0
CIII	3	1	3	0	1	0	3	2	2	2	3	3	1
CSII	1	3	0	3	3	3	0	2	2	2	0	1	3
CR	2	2	2	2	2	2	2	0	1	1	2	2	2
CTI	2	2	2	2	2	2	2	1	0	0	2	2	2
CTII	2	2	2	2	2	2	2	1	0	0	2	2	2
CSIII	1	3	0	3	3	3	0	2	2	2	0	1	3
CB	1	3	1	3	3	3	1	2	2	2	1	0	3
CKII	3	1	3	1	0	1	3	2	2	2	3	3	0

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.

Recently, Guillot and Rousset (2011) reported that partial Mantel tests may suffer from bias in cases where there is spatial correlation in landscape resistance. They suggest that Mantel tests should not be used in case auto-correlation is suspected in both variables. Similarly, Meirmans (2012) argued that spatial autocorrelation deriving from isolation by distance bias the outcome of Mantel tests, leading to a large number of false positives. Amos et al. (2012) reported a similar pattern of results for alternative resistance models rather than isolation by distance.

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 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 (Fig. 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 20 alternative resistance hypotheses (18 WorldClim climate hypotheses, Phylogenetic Distance, and Isolation by Distance). We then selected the few alternative hypotheses that showed the highest support relative to the other resistance models and further evaluated their support relative to each other. In this we formally compared the values of the two partial Mantel tests. For a hypothesis to be supported, the first test should produce a positive Mantel correlation and the second should produce no correlation or a negative Mantel correlation. We evaluated which of the selected hypotheses had the most support relative to the other well supported hypotheses.

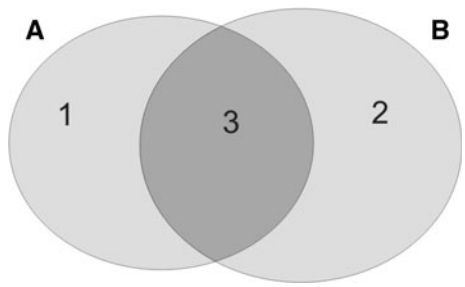

Fig. 2 Support for hypothesis A relative to hypothesis B is determined using two partial Mantel tests. Test 1: G ~ A|B, produces correlation 1 in the diagram above. Test 2: G ~ B|A, produces correlation 2 in the diagram above. If hypothesis A is supported relative to hypothesis B we would expect the value of 1–2 to be positive. In contrast, if hypothesis A is not supported relative to hypothesis B then we would expect the value of 1–2 to be negative

Results

We found that four hypotheses had strong support based on the matrix of difference in support between partial Mantel test 1 and partial Mantel test 2 (Fig. 3). Specifically, phylogenetic distance (PD), geographical distance (ED), interseasonal variability in precipitation (G15), and winter precipitation (G19) were well supported compared to nearly all alternative hypotheses (positive values in columns associated with these hypotheses in Fig. 3). In contrast, none of the other 16 alternative resistance hypotheses were well supported compared to the majority of other hypotheses, and none were well supported relative to PD, ED, G15, G19 (negative or zero values in the rows associated with these hypotheses in Fig. 3).

Accordingly, we evaluated these four well supported hypotheses relative to each other by comparing the partial Mantel r values for Tests 1 and 2 (Table 7). For a hypothesis to be fully supported in Test 1 it should have positive partial Mantel values in each test. PD and winter precipitation (G19) met this criterion, while geographic distance and interseasonal variability in precipitation did not. These latter two models had no support after partialling out winter precipitation, suggesting that they may be spurious correlates and that winter precipitation is the only independently supported hypothesis.

Table 7 Results of partial Mantel tests 1 and 2 for all combinations of the four alternative hypotheses

	PD	ED	G15	G19
Test 1				
PD	X	**0.1408**	**0.1412**	**0.1499**
ED	**0.2724**	X	**0.0272**	**0.0663**
G15	**0.2718**	**0.0151**	X	**0.0783**
G19	**0.2698**	−0.0132	−0.0493	**X**
Test 2				
PD	X	0.2724	0.2718	0.2698
ED	0.1408	X	0.0151	**−0.0132**
G15	0.1412	0.0272	X	**−0.0493**
G19	0.1499	0.0663	0.0783	X

Bold values indicate outcomes that are consistent with expectations of reciprocal causal modeling (Cushman et al. 2013)

For a hypothesis to be fully supported in Test 2, it should have zero or negative partial Mantel values in each test. None of the alternative hypotheses met this expectation. Geographic distance, winter precipitation and variability of precipitation all had large positive correlations with genetic distance independent of

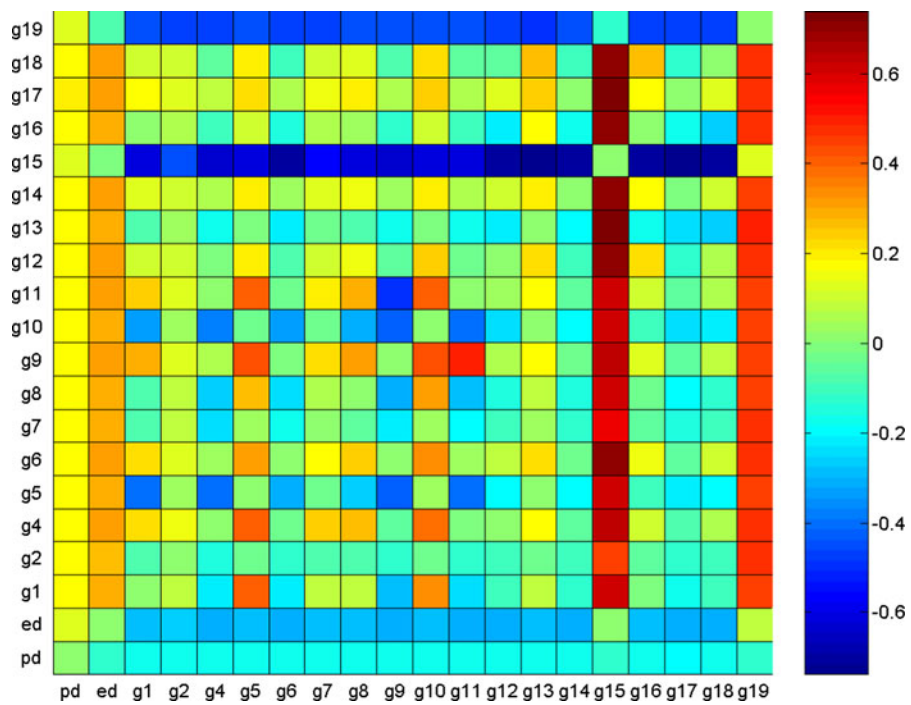

Fig. 3 Matrix of difference in support for *column* hypotheses relative to *row* hypotheses. The values in the table are the difference in partial Mantel r for test 1 (genetic distance ~ column hypothesis | row hypothesis) and test 2 (genetic distance ~ row hypothesis | column hypothesis). Positive values indicate support for the *column* hypothesis relative to the *row* hypothesis

phylogenetic distance (Table 7). This suggested that phylogenetic distance is not a sufficient explanation, by itself, of genetic differentiation. Similarly, geographical distance and variability of precipitation were not supported given that phylogenetic distance and winter precipitation had positive correlations with genetic distance independent of them. Finally, results of Test 2 indicated that winter precipitation was supported independently of geographical distance and variability in precipitation, given the negative partial Mantel correlations in Test 2, but that there remained a large positive correlation with phylogenetic distance independent of winter precipitation.

The combination of results of Tests 1 and 2 suggested three things. First, following Test 1, there was independent support for both phylogenetic distance and winter precipitation as explanations of genetic differentiation among the study populations. Second, following Test 1, geographical distance and variability in seasonal precipitation were not supported, given lack of relationship with genetic distance independent of winter precipitation. Third, Test 2 suggested that neither phylogenetic distance nor winter precipitation is a sufficient independent explanation. This suggests that gene flow among the study populations is jointly related to both phylogenetic distance among populations and the cumulative difference in winter precipitation between them. It further indicates that gradients in winter precipitation are not sufficient to explain all the genetic differences among populations that are related to phylogenetic difference.

Discussion

We found that the amount of winter precipitation influenced genetic differentiation of *Caragana* populations in the Ordos Plateau of northern China. The genetic differentiation in the populations of genus *Caragana* was also related to phylogenetic distance, with no independent relationship with geographical distance. This suggests that the primary factor driving genetic differentiation both within and among *Caragana* species has been variation in climate, specifically spatial variation in the amount of winter precipitation. This suggests that gradients of rapid change in winter precipitation have acted as highly resistant zones that create attenuated gene flow, enabling genetic differentiation of populations and perhaps providing sufficient parapatric conditions to allow differential local directional selection to result in speciation within the *Caragana* clade (Wright 1932; Mayr 1954; McKinnon et al. 2004; Doebeli et al. 2005).

Our study results indicated that variation in precipitation is a much stronger predictor of genetic differentiation among populations and species of *Caragana* than variation in temperature. This suggested that gradients of changing precipitation acted as resistant factors limiting gene flow, and provide stronger prediction of genetic differentiation than isolation by distance. For example, genetic differentiation of the three populations of *C. stenophylla* (CSI, CSII and CSIII) distributed in typical steppe, desert steppe and desert steppe was strongly correlated with patterns of differential winter precipitation, independently of geographical distance, while there was no independent relationship between genetic differentiation and distance, independent of winter precipitation. The geographical distance between CSI and CSIII was the largest among any pairs of populations in this study, but the genetic distance between was the minimum (0.0527). Conversely, while the geographical distance between CSII and CSIII was the minimum, the genetic distance between this pair of populations was the largest observed in this study (0.0774). The most significant environmental difference along the three vegetation zones where *C. stenophylla* (CSI, CSII and CSIII) occurs is the difference of precipitation, and the genetic structure of *Caragana* populations was strongly influenced by precipitation.

Gene flow in plants is mediated by both seed and pollen dispersal, which vary greatly among species (Ennos 1994; Kremer et al. 2012). Generally, 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. 2012).

In the Ordos Plateau, there is very little precipitation in spring. Therefore, differences in winter 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 winter precipitation will influence pollination and seed diffusion, and drive differential patterns of gene flow, and could lead to sufficient reduction in gene flow to enable speciation due to accumulation of genetic incompatibilities (Gavrilets et al. 2000; Hoelzer et al. 2008). In addition, the differential climatic conditions across the precipitation gradient likely impose marked local directional selection on the local populations. These climatic differences likely reduce fitness of locally maladapted individuals, resulting in population divergence and maintenance of reproductive isolation (Gavrilets 2000; Gavrilets and Vose 2007; Niemiller et al. 2008; Nosil 2008; de León et al. 2010).

Our results also found that there was a strong, residual correlation between phylogenetic distance and genetic differentiation among populations after accounting for the effects of winter precipitation. There are several potential explanations for this. First, the *Caragana* taxa included in this study do not fit the biological species concept, which defines species based on physical or physiological reproductive incompatibility. These species can and do hybridize when in proximity. This suggests that the existence of genetic structure associated with phylogenetic differences but not with environmental gradients driving gene flow is likely a relic of previous allopatry during which the taxa genetically diverged. Alternatively, the observation of residual genetic differentiation associated with phylogenetic differences may indicate that other environmental factors in addition to gradients in winter precipitation are in part responsible for driving spatial patterns of gene flow among *Caragana* taxa across the study area. Most importantly, the results clearly indicate that gradients of winter precipitation are highly related to genetic differentiation within and among *Caragana* species, suggesting substantial interspecies gene flow among these closely related congeneric species. Taxonomic boundaries are blurry, and are apparently maintained by limited gene flow across steep gradients of differential winter precipitation.

Among *Caragana* populations on the Ordos Plateau, we believe it is likely that a combination of reduced gene flow driven by differential timing of reproduction driven by differences in seasonal precipitation patterns (Feder et al. 1993; Yamamoto and Sota 2009) coupled with local directional selection (Niemiller et al. 2008; Nosil 2008) led to population differentiation, speciation and maintenance of phylogenetic separation of *Caragana* populations. It is likely that this genetic differentiation is driven by phenological differences between populations in areas with different patterns of winter precipitation, such that timing of flowering may be asynchronous between such populations, greatly limiting gene flow, and enabling peripatric speciation (Mayr 1954) within the adaptive landscape (Wright 1932).

Acknowledgments This work was supported by Science Foundation of Ministry of Science and Technology of China (2011BAC07B01).

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