Plant Syst Evol
DOI 10.1007/s00606-015-1196-z

ORIGINAL ARTICLE

Inferring ancestral distribution area and survival vegetation of *Caragana* (Fabaceae) in Tertiary

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Received: 1 September 2014 / Accepted: 7 January 2015

Abstract *Caragana*, a leguminous genus mainly restricted to temperate Central and East Asia, occurs in arid, semiarid, and humid belts, and has forest, grassland, and desert ecotypes. Based on the previous molecular phylogenetic tree and dating, biogeographical analyses of extant species area and ecotype were conducted by means of four ancestral optimization approaches: S-DIVA, Lagrange, Mesquite, and BBM. The results indicate the ancestral attributes of *Caragana* as an arid origin from the Junggar Basin and arid belt of climate and vegetation in the middle Miocene. The ancestral ecotype was most likely adapted to steppe habitats. Uplift and expansion of the Qinghai-Xizang (Tibet) Plateau (QTP) and retreat of the Paratethys Sea are believed to have led to this origin, and also the subsequent diversification and adaptive radiation in the genus. The direction of radiation is suggested in brief to have been from the Central Asian Junggar to East Asia and Tibet.

Keywords Biogeography · Temperate Asia · Arid origin · Adaptive radiation · Spatial evolution · Miocene

Handling editor: Yunpeng Zhao.

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Introduction

The genus *Caragana* (Fabaceae) comprises approximately 100 species belonging to five sections (Liu et al. 2010; Zhang 1997a; Zhang et al. 2009; Zhao 2008), which are mostly native to temperate Asia. This genus is attractive because of the obvious morphological differences involving leaflet arrangement (either pinnate or palmate) and the rachis (either deciduous or persistent; Moore 1968), and because of floristic implications relating to its distribution, and the details of its origin and evolution. The genus has been studied from many aspects, relating to macro-morphology and classification, chromosome number, pollen morphology, molecular phylogeny, molecular dating, and analytical biogeography.

Komarov (1908) published the first monograph of *Caragana*, in which eight series were delimited. On the basis of morphological variation and distribution patterns, he (Komarov 1908, 1947) used *Caragana* and four other genera to hypothesize floristic connections between China and Mongolia. He suggested that *Caragana* originated in East Asia, probably eastern China, with *C. sinica* (Buc'hoz) Rehder, which has only two pairs of leaflets, as the most primitive species. However, based on chromosomal evidence, Moore (1968) rejected the East Asian origin, since *C. sinica* is triploid with a chromosome number of $2n = 24$. Instead, he inferred a Central Asian origin, specifically near southern Lake Balkhash, the Tianshan Mts., and adjacent

Published online: 24 January 2015

Mongolia, where most series of the genus can be found. Sanchir (1979) and Zhao (1993) proposed *C. arborescens* Lam., with numerous pairs of pinnate leaflets, a deciduous rachis, a diploid chromosome number of $2n = 16$, and a temperate forest distribution, as the ancestral species of the genus instead of *C. sinica*. Similar conclusions have been reached as a result of analytical approaches such as component ancestral area, and dispersal and vicariance analyses (Zhang 1998, 2004, 2005).

Recently, we conducted a molecular phylogenetic analysis based on three genic regions for *Caragana* (Zhang et al. 2009). Three strongly supported major clades were recovered, corresponding to three of the five sections within the genus, i.e. sections *Caragana, Frutescentes*, and *Bracteolatae* (Zhang 1997b). Then, we examined the historical biogeography of the genus employing molecular dating approaches (Zhang and Fritsch 2010). The results suggested that early Meiocene uplift of the QTP (Harris 2006; Shi et al. 1998, 1999; Li and Fang 1998) appears to have coincided with the origin of *Caragana*, while origin of the three stable sections within the genus occurred during expansion of the QTP, following the mid-Miocene (Li et al. 2011), in concert with increasing aridification of the Asian interior during that time.

As mentioned above, *Caragana* molecular phylogeny and temporal dating have been carried out previously; however, analysis of its spatial biogeography is treated more intensively in this paper, especially investigation of the origin and evolution of distribution areas, and ecological adaptation and geographical diversification, which had only been preliminary hypotheses based on morphological variation (Zhang 1998, 2004, 2005). Four ancestral optimization methods: S-DIVA (Nylander et al. 2008; Yu et al. 2010), maximum likelihood (ML) statistical model Lagrange (Ree and Smith 2008), Fitch parsimony optimization (FPO) Mesquite (Maddison and Maddison 2009), and Bayesian Binary Method (BBM) (Yu et al. 2010) are employed to associate spatiotemporal diversification with the geological framework (e.g. Antonellia et al. 2009; Bendiksby et al. 2010; Couvreur et al. 2011; Emadzade and Horandl 2011; Greve et al. 2010; Thiv et al. 2010; Gussarova et al. 2008; Pepper et al. 2011; Sarnat and Moreau 2011; Spalik et al. 2010). At the same time, adaptive radiation (Givnish and Systma 1997; Sanderson 1998; Linder 2008; Glor 2010) as a speciation process in *Caragana* across the QTP and adjacent areas, with a characteristic of rapid species divergence during the middle and later Miocene, should be associated with the nature of the paleoclimate and paleovegetation (e.g. Morley 2003; Linder 2008; Lavergne et al. 2010).

Concerning the vegetation and climate of the Cenozoic in Asian temperate regions of the *Caragana* distribution, QTP uplift has had a particularly important influence (e.g. Quade et al. 1989; Tao 1992; Coleman and Hodges 1995; Zhong and Ding 1996; Li and Fang 1998; Shi et al. 1998,1999; An et al. 2001; Guo et al. 2008). Another important geological event to be related to *Caragana* evolution would be Paratethys withdrawal westward in the Oligocene–Paleogene (Zhang et al. 2007; Ramstein et al. 1997).

Therefore, the goal of this paper is to focus on: (1) inference of ancestral attributes of the genus, and subsequent diversifications, by means of spatial biogeographical analysis, and employing data of distribution area and ecotype; (2) coupled with the framework of vegetation and climate of the Cenozoic, to discuss spatiotemporal hypotheses and ancestral attributes of *Caragana*, in other words, whether or not the suggested ancestral attributes of *Caragana* are consistent with the paleovegetational and paleoclimatic evidence.

Materials and methods

Phylogenetic tree

The phylogenetic tree is from our previous study, based on the three sequences *rbcL, trnS–trnG*, and ITS (Zhang et al. 2009; Fig. 3), which is the best developed cladogram for *Caragana* so far, and so appropriately used as a base of biogeographical analysis. In addition, we also obtained a BEAST dating tree (Zhang and Fritsch 2010; Fig. 2) whose topology is different. Therefore, it is also used as a basis for biogeographical comparison. Constructions of these two trees are described in detail in the previous papers (Zhang et al. 2009; Zhang and Fritsch 2010). For both of them as used in the present study, outgroups of *Astragalus* and *Hedysarum* were included in the biogeographical analyses, although not shown in the resulting figures.

Distribution areas of *Caragana*

Essentially, the *Caragana* distribution range pertains to the floras of East Asia and Central Asia, and most of the species appear in China. Thus, data of the flora, vegetation, climate, paleoenvironment, and paleogeography of China were employed in the analysis.

The division into areas of the *Caragana* distribution in this paper is mainly on the basis of floristics (Grubov 1999; Wu and Wu 1998), vegetation (Wu 1980; Tao 1992; Song et al. 1983; Guo 1983; Willis and McElwain 2002), and climate (Tao 1992; Willis and McElwain 2002; Guo et al. 2008), in combination with our previous division of the genus (Zhang 1998, 2004). The distribution can be divided into two parts: East Asia and Central Asia (Zhang 1998). The East Asian part consists mainly of Far East–northeastern China, northern China, the Hengduan Mts., and the

eastern Himalayas (Wu and Wu 1998), all with humid forest vegetation (Wu 1980). Additionally, Tibet can be included in East Asia (Wu and Wu 1998). The Central Asia distribution according to Grubov (1999) and Wu and Wu (1998) may be separated into three areas, eastern Mongolia with semi-humid steppe, Kashchgaria including western Mongolia and the Tarim Basin with arid desert, and the Junggar Basin, including Junggar–Turan, with arid desert.

Finally five areas are defined, i.e. A: East Asia, B: eastern Mongolia, C: Kashgar, D: Junggar, and E: Tibet, see Figs. 1, 3. Species distributions and their assignments to these five areas are shown in Tables 1 and 2 and Figs. 1 and 3.

Ecotypes of *Caragana*

Caragana has distinctive species occurring in a variety of vegetation zones. Species of the genus can appear in alpine meadow, forest, grassland, or desert, and are often the most dominant species in the community, responsible for its peculiar vegetation formations, particularly in grassland and desert (Wu 1980). For instance, Formation *C. korshinskii* is found in western Mongolia, Form. *C. tibetica* in Tibet and western Mongolia, and Form. *C. acanthophylla* on the northern slopes of the Tianshan Mts. To explore the ancestral attributes and evolution of *Caragana* ecotypes, six ecotypes of extant species, A: forest, B: steppe, C: desert, D: alpine, E: sub-alpine, and F: shrub, were defined, and are shown in Tables 1 and 2, and Figs. 2 and 4. These, as well as other ancestral attributes, such as life form, dispersal mode, habit history, and insect forms hosted by the plant (e.g. Winkler et al. 2009; Bytebier et al. 2011; Xiang et al. 2014), can be inferred from these categories.

Optimization of ancestral distribution

To infer ancestral area character and vicariance and dispersal events, four approaches were used: statistic DIVA

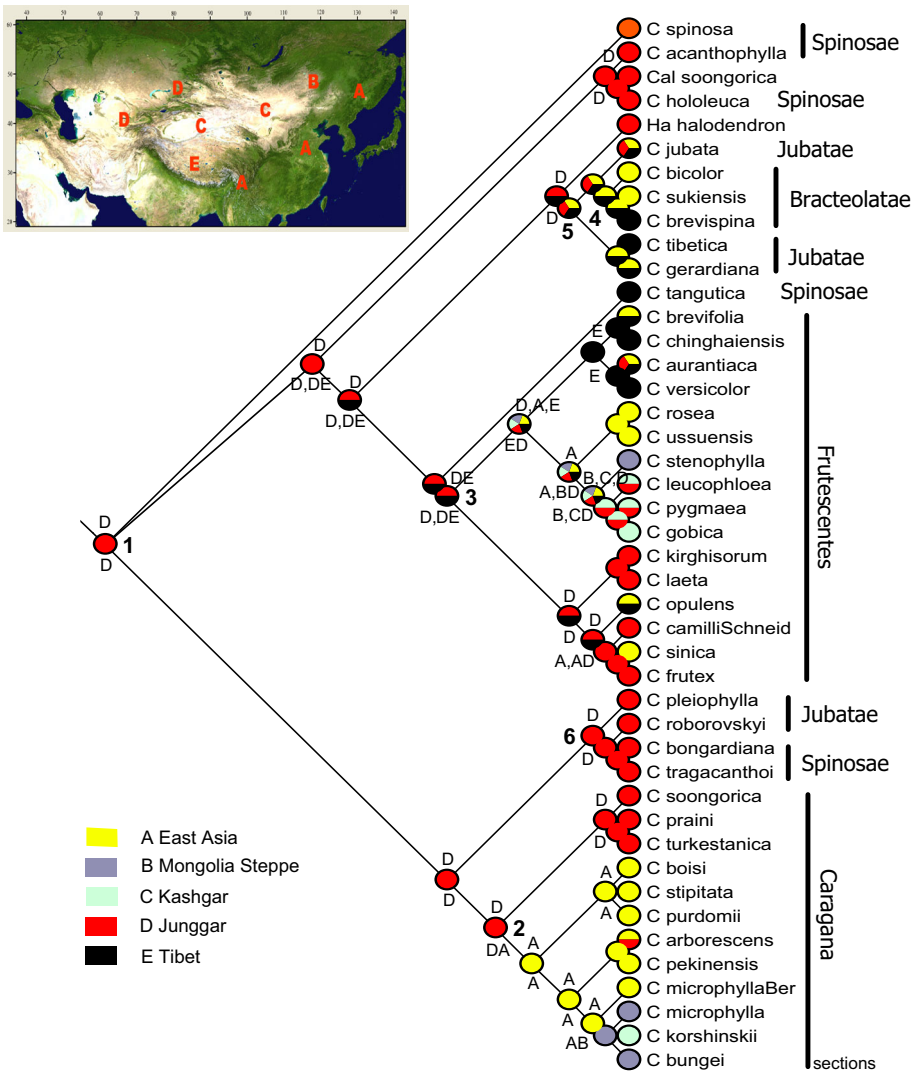


Fig. 1 Biogeographical ancestral optimization of *Caragana* areas, conducted for tree 1 using the approaches Mesquite, BBM and Lagrange, based on the combined 3-gene data set (Zhang et al. 2009, Fig. 3). *Shading* shows ancestral area reconstruction under parsimony in Mesquite. MRCA areas reconstructed by BBM are marked above at each node, and those by Lagrange below. Detailed information can be found in Table 2

Table 1 Distribution areas and ecotypes of 48 species of *Caragana* (49 samples) and outgroup genera

Taxon	Distribution area	Ecotype
Sect. *Caragana*		
Ser. *Caragana*		
C. arborescens Lam.	AD	A
C. boisii C.K.Schneid.	A	A
C. prainii C.K.Schneid.	D	B
C. purdomii Rehder	A	A
C. soongorica Grubov	D	BF
C. stipitata Kom.	A	A
C. turkestanica Kom.	D	AF
C. zahlbruckneri C.K.Schneid.	A	A
Ser. *Microphyllae* (Kom.) Pojark.		
C. bungei Ledeb.	B	B
C. korshinskii Kom.	C	BC
C. microphylla Lam.1	AB	AB
C. microphyllaBer Lam.2	AB	AB
C. pekinensis Kom.	A	A
Sect. *Bracteolatae* (Kom.) M.L.Zhang		
Ser. *Bracteolatae* Kom.		
C. bicolor Kom.	A	ADF
C. brevispina Benth.	E	AD
C. franchetiana Kom.	A	ADF
C. sukiensis C.K.Schneid.	A	AD
Ser. *Ambiguae* Sanchir		
C. ambigua Stocks	E	BD
C. conferta Benth. ex Baker	E	BD
Sect. *Jubatae* (Kom.) Y.Z.Zhao		
Ser. *Jubatae* Kom.		
C. jubata (Pall.) Poir.	ADE	ADE
C. pleiophylla (Regel) Pojark.	D	BCE
C. roborovskyi Kom.	D	C
C. tangutica Maxim.	E	AEF
Ser. *Leucospinae* Y.Z.Zhao		
C. changduensis Y.X.Liou	A	DE
C. gerardiana Benth.	AE	DE
C. tibetica (Maxim. ex C.K.Schneid.) Kom.	CD	BCE
Sect. *Frutescentes* (Kom.) Sanchir		
Ser. *Frutescentes* Kom.		
C. camilli-schneideri Kom.	D	B
C. frutex (L.) K.Koch	D	AB
C. kirghisorum Pojark.	D	B
C. laeta Kom.	D	B
C. opulens Kom.	AE	BDE
C. polourensis Franch.	C	C
Ser. *Chamlagu* Pojark.		
C. rosea Turcz. ex Maxim.	A	A
C. sinica (Buc'hoz) Rehder	A	A
C. ussuriensis (Regel) Pojark.	A	A
Ser. *Pygmaeae* Kom.		
C. aurantiaca Koehne	ADE	BDE
C. brevifolia Kom.	AE	BE
C. chinghaiensis Y.X.Liou	E	AD
C. gobica Sanchir	C	BC
C. leucophloea Pojark.	CD	BC
C. pygmaea (L.) DC.	CD	BC
C. stenophylla Pojark.	B	B
C. versicolor Benth.	E	D
Sect. *Spinosae* (Kom.) Y.Z.Zhao		
Ser. *Spinosae* Kom.		
C. bongardiana (Fisch. & C.A.Mey.) Pojark.	D	B
C. bongardiana (Fisch. & C.A.Mey.) Pojark. 1	D	B
C. hololeuca Bunge ex Kom.	D	B
C. spinosa (L.) Hornem.	D	B
C. tragacanthoides (Pall.) Poir.	D	B
Ser. *Acanthophyllae* Pojark.		
C. acanthophylla Kom.	D	B
Ser. *Dasyphyllae* Pojark.		
C. dasyphylla Pojark.	D	B
C. dasyphylla Pojark. 1		
Outgroups		
Calophaca soongorica Kar. & Kir.	D	B
Calophaca soongorica Kar. & Kir. 1		
Halimodendron halodendron (Pall.) Voss.	D	B
Hedysarum alpinum L.		
Astragalus coluteocarpus Boiss.		

The classification of *Caragana* follows Zhang (1997)

Five distribution areas of *Caragana*: *A* East Asia, *B* eastern Mongolia *C* Kashgar, *D* Junggar, *E* Tibet

Six ecotypes: *A* forest, *B* steppe, *C* desert, *D* alpine, *E* sub-alpine, and *F* shrub

(S-DIVA, RASP v.2.1b Yu et al. 2010, http://mnh.scu.edu.cn/soft/blog/RASP), a maximum likelihood-based method Lagrange v.2.0.1 (Ree and Smith 2008), a Fitch parsimony optimization implemented in Mesquite v.2.6 (Maddison and Maddison 2009), and the Bayesian Binary Method (BBM) implemented in RASP.

In this paper, we reconstruct MRCA (most recent common ancestor) at the nodes of the phylogenetic tree and also of ecotype as well.

Table 2 Biogeographical analyses of combined 3-gene sequences and two phylogenetic trees, tree 1 (Zhang et al. 2009, Fig. 3) and tree 2 (Zhang and Fritsch 2010, Fig. 2) concerned

Beast				Area analyses of four approaches			Ecotype analyses of four approaches			
Node	Taxa	Mean	95 % HPD	Mesquite	S-DIVA	BBM	Lagrange	Mesquite	S-DIVA	BBM	Lagrange
1	Genus *Caragana*	16.15	9.96–20.34	T1: D	T2: D, DE	T1: D	T1: D	T1: B	T2: B	T1: B	T1: B
						T2: D	T2: D, AD			T2: B	T2: B, AB
2	Sect. *Caragana*	7.99	4.08–12.62	T1: D	T2: AD	T1: D	T1: DA	T1: B	T2: AB	T1: B, AB	T1: A, AB
						T2: D	T2: DA			T2: B	T2: B, AB
3	Sect. *Frutescentes*	7.49	2.52–12.63	T1: DE	T2: E	T1: DE	T1: D, DE	T1: B	T2: B	T1: B	T1: B
						T2: E	T2: E, AE			T2: B	T2: B
4	Sect. *Bracteolatae*	4.45	0.75–8.54	T1: AE	T2: AE	T1: A	T1: A	T1: AD	T2: D	T1: AD	T1: D, A
						T2: E, A	T2: AE			T2: BD,D	T2: BD, D
5	Sect. *Jubatae* + *Bracteolatae*	7.63	1.22–14.3	T1: ADE	T2: E, AD	T1: DE, ADE	T1: ADE, E	T1: ABDE	T2: D,DE	T1: D,BD	T1: D, BD
						T2: D, DE	T2: AE, AD			T2: BD,D	T2: BD, D
6	Sect. *Jubatae* + *Spinosae*	5.46	1.00–15.08	T1: D	T2: D	T1: D	T1: D	T1: B	T2: B	T1: B	T1: BCE, B, C
						T2: D	T2: D, AD			T2: B	T2: B

BEAST dating of combined 3 gene sequences comes from our previous analysis (Zhang and Fritsch 2010), nodes refer to tree 2

A–E in Area analysis and A–F in Ecotype analysis refer to Table 1. Approaches Mesquite, BBM, and Lagrange are used to tree 1, whereas S-DIVA, BBM, and Lagrange to tree2. Constrained maximum areas and ecotypes in S-DIVA are with maxarea 2

S-DIVA

S-DIVA (Bayes-DIVA), based on DIVA, calculates the posterior distribution of a Bayesian MCMC sample of tree topologies (Nylander et al. 2008). S-DIVA is performed in RASP (Reconstruct Ancestral State in Phylogenies) 2.0 beta. http://mnh.scu.edu.cn/soft/blog/RASP (Yu et al. 2010). The two trees (Zhang et al. 2009, Fig. 3, Zhang and Fritsch 2010, Fig. 2) were each treated as a fully resolved phylogram for use as a basis for S-DIVA, with 711 post-burnin trees derived from the Beast analysis employed for ancestral area reconstruction in the program RASP, in which various constraints of maxareas = 2 at each node were used to infer possible ancestral areas and potential vicariance and dispersal events.

BBM

BBM (Bayesian Binary Method) infers ancestral area using a full hierarchical Bayesian approach (Ronquist 2004) and hypothesizes a special "null distribution", meaning that an ancestral range contains none of the unit areas. BBM is implemented in RASP with default option. Fixed JC + G (Jukes-Cantor + Gamma) were used for BBM analysis with a null root distribution.

Lagrange

A valuable, newly developed biogeographical methodology is parametric likelihood analysis, with a dispersal–extinction–cladogenesis model (Ree and Smith 2008), as implemented in Lagrange v. 2.0.1 (Ree and Smith 2008). This methodology calculates the likelihood of biogeographical routes and areas occupied by the MRCA for a given phylogenetic tree topology and the present distributions of taxa. Therefore, dispersal and vicariance of lineages, represented by the connection areas, can be estimated by the probabilities. This is a form of MRCA area reconstruction different from the parsimony approach of DIVA and S-DIVA. The two trees (Zhang et al. 2009; Fig. 3; Zhang and Fritsch 2010; Fig. 2) were used as an analytical base.

Mesquite

Reconstruction of ancestral states was based on FPO implement by Mesquite (Maddison and Maddison 2009). Fitch parsimony calculates the most parsimonious ancestral states at the nodes of the tree, assuming one step per state change. In general the FPO assumes that geographical distributions are the result of dispersal events rather than vicariance. The primary phylogenetic tree (Zhang et al. 2009; Fig. 3) was used as the analytical base.

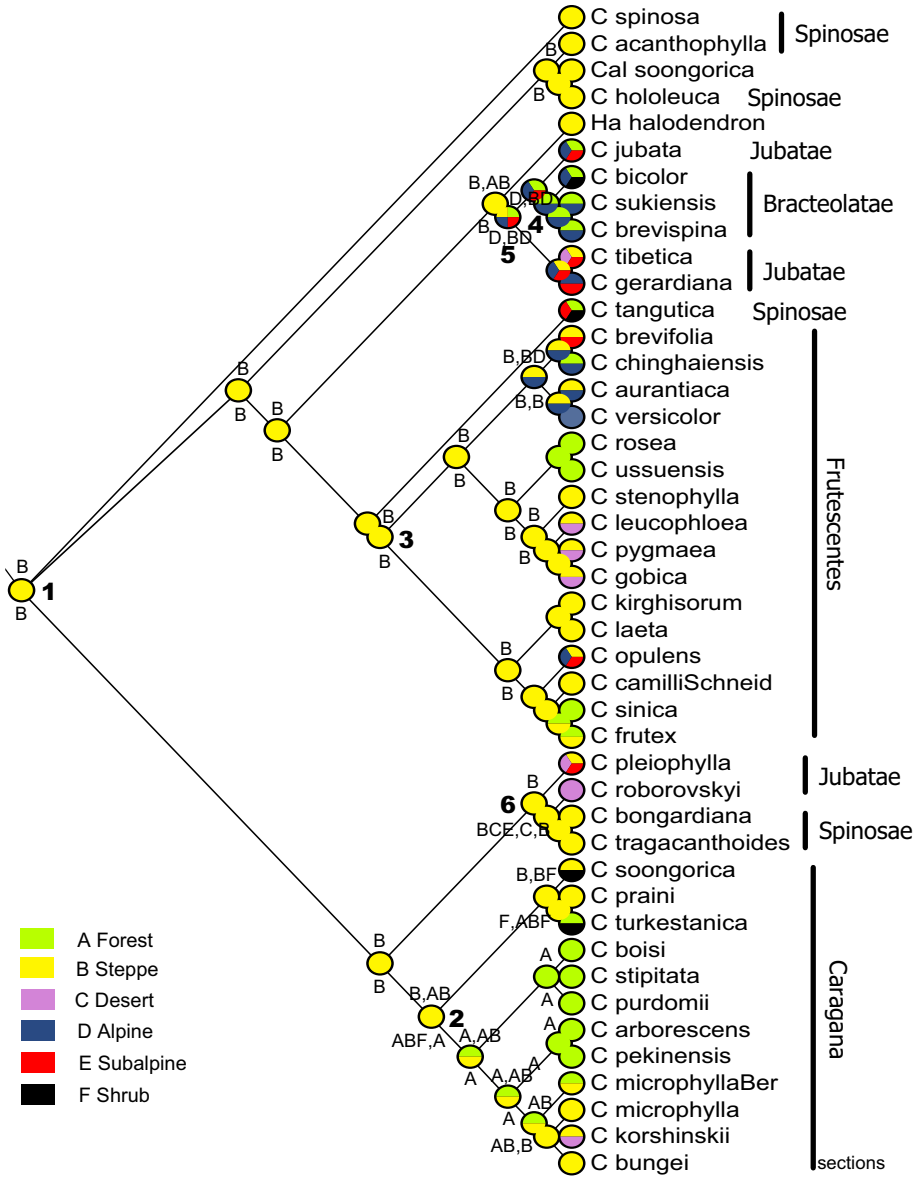


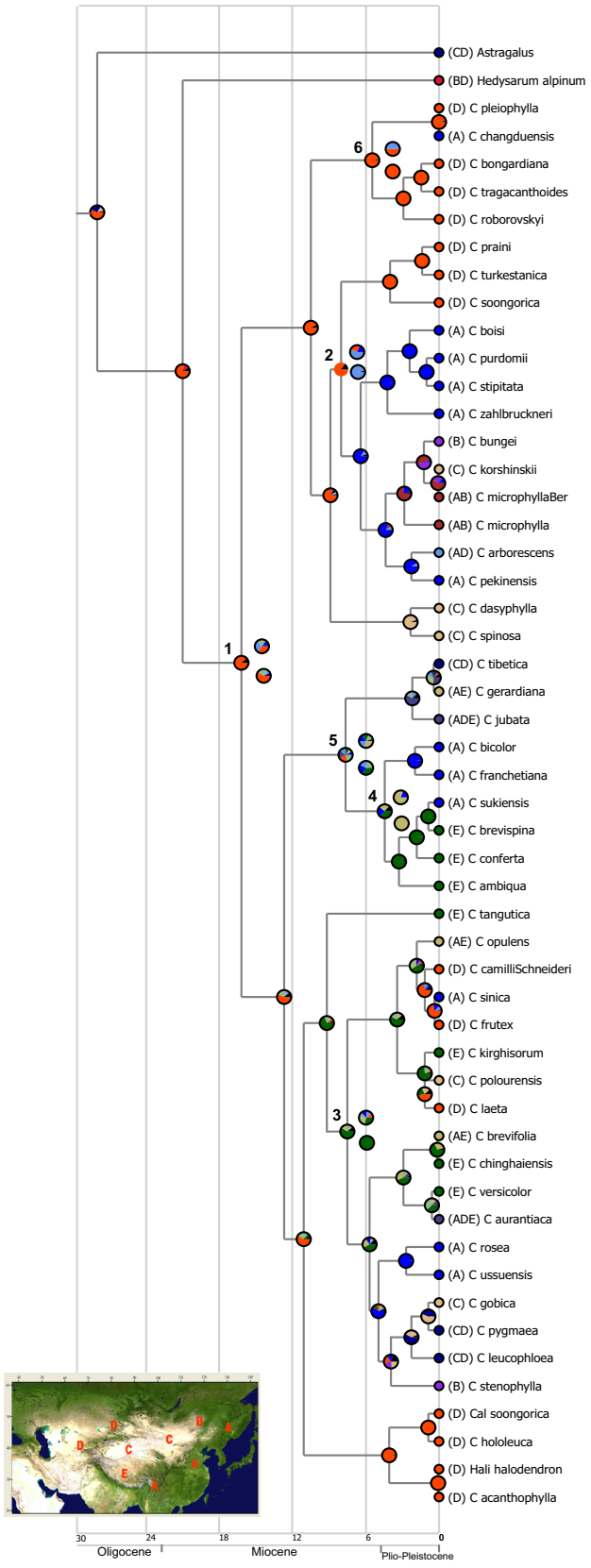
Fig. 2 Biogeographical ancestral optimization of *Caragana* species ecotypes, conducted for tree 1 using the approaches Mesquite, BBM and Lagrange, based on the combined 3-gene data set (Zhang et al. 2009, Fig. 3). *Shading* shows ancestral area reconstruction under parsimony in Mesquite. MRCA ecotypes as reconstructed by S-DIVA are indicated above at each node, and by Lagrange below. Detailed information can be found in Table 2

Results

Based on the biogeographical basis of the two trees mentioned above, four approaches, Lagrange, Mesquite, S-DIVA, and BBM, were employed to reconstruct ancestral states of species areas and ecotypes. In view of the differences of topology of the trees, it might be expected that the ancestral reconstructions of species areas (or ecotypes) would be different. However, a rough similarity of node estimates between the two and among approaches was observed, especially at the six nodes of genus and sections; see Figs. 1, 2, 3, 4 and Table 2. Concerning node estimation of the two trees using Lagrange, there is resemblance of estimates at the six corresponding nodes, see Table 2. These results of comprehensive comparisons between two trees and among four approaches have the advantage of enhancing the creditability of the species area and ecotype estimates.

Area analysis

Biogeographical ancestral area analyses of tree 1 (Zhang et al. 2009; Fig. 3) by Mesquite, BBM, and Lagrange is seen in Fig. 1, and of tree 2 (Zhang and Fritsch 2010;

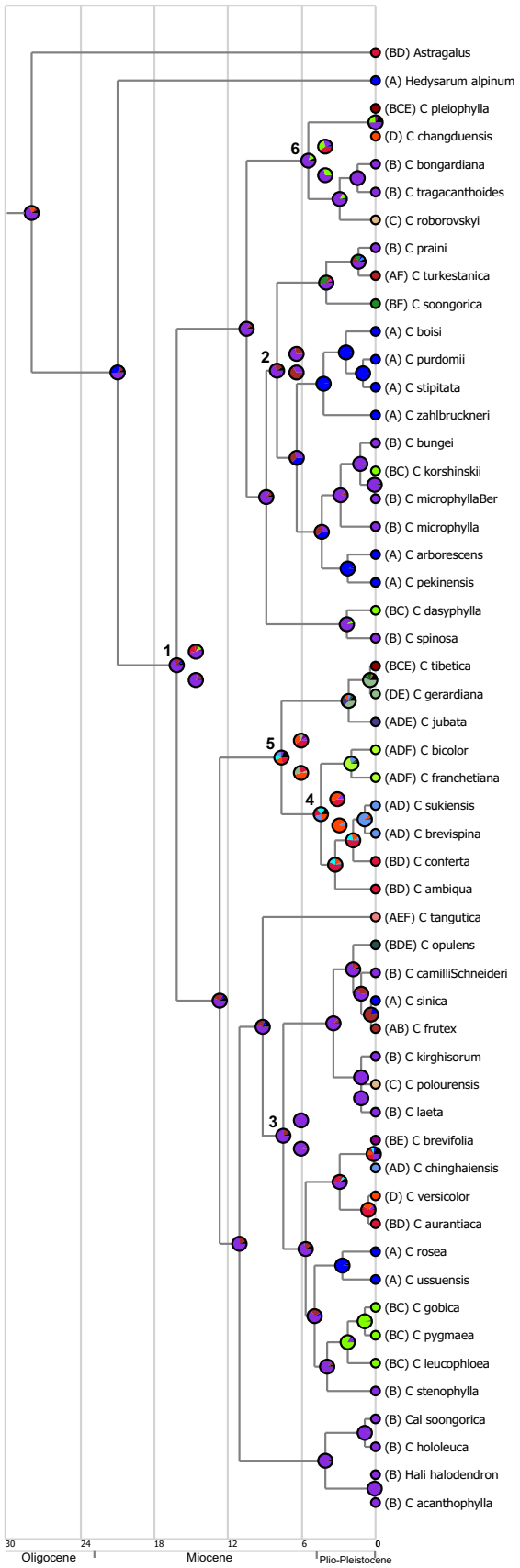


◀**Fig. 3** Biogeographical ancestral optimization of *Caragana* areas, conducted for tree 2 using the approaches BBM, S-DIVA, and Lagrange, based on the combined 3-gene data set (Zhang and Fritsch 2010, Fig. 2). Pie charts at nodes show ancestral area reconstruction under BBM. MRCA areas reconstructed by Lagrange are marked above at the *right* of each node, and those by S-DIVA below *right*. Detailed information can be found in Table 2

Fig. 2) by S-DIVA, BBM, and Lagrange in Fig. 3, have a congruent pattern of MRCA and vicariance and dispersal for several important nodes. The ancestor of the genus *Caragana* (see Figs. 1, 3; Table 2) is fairly placed at Junggar (D) by all of the methods, and the MRCA areas for the sections *Jubatae* and *Spinosae* at node 6 are mostly Junggar (D), illustrating that dispersals are from the ancestral Junggar Basin. The MRCA area of section *Caragana* at node 2 is shown as a combination of Junggar and East Asia (AD) in S-DIVA and Lagrange, but D in Mesquite and BBM, A should be a dispersal from D. The MRCA area of section *Frutescentes* is likely DE Junggar (D) and Tibet (E) or Tibet (E). Thus, taxa in Junggar (D) could be regarded as the diversifications autochthonic, whereas those in Tibet (E) could be regarded as dispersals. Based on the estimated results, because a vicariance occurs for section *Caragana* between Junggar and East Asia, the former has *C. soongarica, C. praini* and *C. turkestanica*, and the latter has most of the other species within the section; we should presume the East Asian distribution to be a dispersal from Junggar. Section *Bracteolatae* at node 4 has a consistent AE (East Asia and Tibet) to be explainable of endemic distribution. On the whole, the biogeographical analyses in Fig. 1 based on tree 1 and Fig. 3 on tree 2 seem consistent, except for uncertainty estimation at node 5 sections *Jubatae* + *Bracteolatae*, which is probably resulted from different tree topologies of Figs. 1, 2 and Figs. 3, 4, different species and distributions, or/and different approaches.

Ecotype analysis

The ancestral root of the genus is consistently steppe (B) as shown in Figs. 2 and 4 and Table 2. At nodes pertaining to sections, the inferred ancestral ecotypes of species are likewise almost all steppe (B), particularly sections *Caragana* (node 2), *Frutescentes* (node 3), *Jubatae* + *Spinosae* (node 6) (with species *C. pleiophylla*, …, *C. tragacanthoides*), *Spinosae*, the exceptions being sections *Bracteolatae* with alpine (D), *Bracteolatae* + *Jubatae* possibly with alpine (D), or forest (A). The node for section *Caragana* was most likely steppe (B), and northern China species such as *C. boisi*, …, *C. pekinensis* (forest A)



◀**Fig. 4** Biogeographical ancestral optimization of *Caragana* species ecotypes, conducted for tree 2 using the approaches of BBM, S-DIVA, and Lagrange, based on the combined 3-gene data set (Zhang and Fritsch 2010, Fig. 2). Pie charts at nodes show ancestral area reconstruction under BBM. MRCA ecotypes reconstructed by Lagrange are marked above at the *right* of each node, and those by S-DIVA below *right*. Detailed information can be found in Table 2

are indicated to be a dispersal from its MRCA ecotype of steppe (B). The alpine and sub-alpine forest ecotypes (A) of sections *Bracteolatae* and *Jubatae* also seem to be dispersals from steppe (B), see Figs. 2 and 4.

Discussion

Arid ancestral attributes of *Caragana*

From our biogeographical analyses, which inferred Junggar as the ancestral location (Figs. 1, 3), and ca. 14–16 Ma (Zhang and Fritsch 2010) as the generic diversification time, we can describe the ancestral attributes of *Caragana* spatiotemporally as follows: the ancestor was living within the steppe vegetation and arid climate belts, and was thus evidently a steppe ecotype. This hypothesis is in accordance with the vegetation and climate of the area in the Cenozoic (Song et al. 1983; Guo 1983; Tao 1992; Willis and McElwain 2002; Guo et al. 2008).

During the Tertiary, a large geographical divergence in paleovegetation and paleoclimate is known to have occurred in China. Based on plant fossils, Tao (1992) suggested a floristic division of the Chinese vegetation, and the current *Caragana* species distribution includes all of her four Neogene floristic regions, namely, temperate forests and grasslands to semi-desert and desert floras of northwestern China, warm temperate deciduous forests of northern and northeastern China, warm temperate to subtropical deciduous and evergreen of forests of central and eastern China, and subtropical evergreen and deciduous forests of Yunnan and the Xizang Plateau. These four floristic regions generally correspond to our previous three areas for *Caragana*: East Asia, the QTP, and Central Asia (Zhang 1997b; Zhang and Fritsch 2010). Our present suggestion of the *Caragana* ancestral vegetation and flora as the Junggar steppe in middle Miocene thus falls into the category of Neogene temperate forests and grasslands of northwestern China sensu Tao (1992), and we can probably say Junggar grassland, since the Junggar Basin at that time could not have been forested.

More accurately, based on the evidence of sporopollen assemblages, Song et al. (1983) divided Miocene China into three floras: the interior forest grassland and grassland

flora (northwestern China), the Qinghai-Xizang (Tibet) *Quercus–Betula*—shrub flora (QTP), and the eastern monsoon broad-leaved flora (northern and southern China of East Asia). Especially, the Junggar area in the Miocene is described as mainly a broad grassland landscape (Song et al. 1983), although with some areas of partly forested grasslands only near the Tianshan and Altai Mts., etc. If our inference of ancestral ecotype had suggested a forest-adapted species, it would have thus conflicted with the Junggar as the biogeographically determined area of origin.

In terms of the Asian paleoclimatic framework outlined by Guo et al. (2008), in the middle Miocene, China is inferred to have had three climate belts, namely, an arid belt, corresponding to northwestern China, a semi-humid and sub-humid belt (near the arid belt) located in western Inner Mongolia and western Gansu provinces, and a humid belt including the southern QTP, and central, northern, and northeastern China. Our inferred paleoclimate of *Caragana* in middle Miocene is in the Junggar arid belt, belonging to the arid belt of northwestern China (Guo et al. 2008). Obviously, the inference of an arid origin for *Caragana* is rationally supported and illuminated by the paleoclimatic framework. Thus, our speculation of the origin and ecotype of *Caragana* in the middle Miocene is in accordance with evidence regarding paleovegetation and paleoflora obtained from fossils and sporopollen assemblages.

Two driving factors for the arid origin and diversification of *Caragana*

In the previous study (Zhang and Fritsch 2010), molecular phylogenetic dating inferred ca. 16 Ma as the crown and ca. 21 Ma as the stem age of the genus, which are heuristically related to QTP uplift in the late Oligocene and early Miocene. Aridification of the Asian interior is generally speculated as resulting from two, not necessarily independent factors, i.e., the retreat of the Paratethys Sea and QTP uplift (Zhang et al. 2007; Ramstein et al. 1997), which strikingly changed the climate of the Asian interior, converting it from humid and coastal to continental and blocking warm and humid airflow from the Indian Ocean.

Hrbek and Meyer (2003) reviewed that the western retreat of the Paratethys took place near the Oligocene/Miocene boundary. From Oligocene ca. 30 Ma to middle-to-late Miocene, the Paratethys shrinkage is hypothesized to have played an important role in transformation of the Central Asian climate from an oceanic to a continental condition (Ramstein et al. 1997). The Junggar area, located at the northern coast of the Paratethys, should have been locally humid in climate during the Oligocene, very similar to Oligocene environments bordering the sea in Kazakhstan, with broad-leaved forest and swamps, and a wet climate (Zubakov and Borzenkova 1990). This humid climate was thereafter replaced by more arid climates concomitant with Paratethys shrinkage, and it appears that the *Caragana* ancestor must have developed in adaptation to these environments. Therefore, identification of an arid Junggar origin for the genus essentially leads us to link Paratethys shrinkage as a major driving force.

Within the genus there is presently a xeric group, particularly section *Frutescentes*, in grassland and desert of Central Asia with the morphological characters of palmate leaflets and a persistent rachis; a cold and xeric group, section *Bracteolatae*, in forest and grassland of QTP with pinnate leaflets and a persistent rachis; and a mesic group, section *Caragana*, in the forests of northern—northeastern China and Junggar with pinnate leaflets and deciduous rachis. These distribution patterns and morphological adaptive variations could be considered as the evolutionary trace and response to environments that also became available because of QTP uplift and the Asian interior aridification process. Therefore, *Caragana* provides a biological case to show evidence for climate change and paleogeographic events in Central Asia and East Asia since early Miocene.

Diversification within *Caragana*

After inferring the *Caragana* ancestral status, we can discuss diversification within the genus. From the biogeographic analysis (Figs. 1, 2, 3, 4; Table 2), we can find many adaptive radiations and dispersals, mainly coming out from the Junggar. Most are so-called mature radiations occurring in the Neogene (Linder 2008). One obvious dispersal event is shown from the ancestral location in the Junggar to East Asia within section *Caragana,* see Figs. 1 and 3.

The East Asian and QTP group, especially sections *Bracteolatae* and *Jubatae*, even though not forming a valid monophyletic group (Zhang et al. 2009) and consequently an unified biogeographical ancestral reconstruction from this paper, we can clearly find that its origin is from the genus MRCA area, the Junggar (see Figs. 1, 3), and its diversifications in situ can well be indicated by the many endemic species. Section *Bracteolatae* is distributed in the Hengduan Mts. and along the Himalayas and westward (Zhang 1997a; Zhang and Fritsch 2010). Most species of section *Bracteolatae* occur in the Hengduan Mts., which belong to the East Asian flora, and are regarded as the distribution center of this section (Zhang 1997b). This section could be speculated to have dispersed into the Himalayas and westward from the Hengduan Mts. Section *Jubatae* occurs in East Asia, Tibet, and Central Asia; it is represented by the most widespread species in the section and genus, *C. jubata.* However, due to the ancestry uncertainty of *C. jubata* and non-monophyly of this section

in the phylogenetic tree (Zhang et al. 2009; Fig. 3, and Figs. 1, 3 in this paper), we could not at present infer certain dispersals and other biogeographical events for this species and section, which will rely on a solid phylogenetic tree in the future.

In contrast to the previously inferred adaptive radiation from humid forest to arid grassland and desert based on morphological characters (Zhang 1998, 2004, 2005; Sanchir 1979; Zhao 1993, 2008), the biogeographic analyses presented here have changed many scenarios of generic evolution, in particular, our determination of an arid origin for *Caragana*. An arid subtropical climate and vegetation existed during the Miocene in the Junggar Basin and northwestern China, and can congruously explain the possibility of this origin. This somewhat agrees with Moore (1968), who considered southern Balkhash Lake, roughly equal to Junggar, which holds different section or series of the genus, as the place of origin and the diversification center. Consequently, as presently updated, Central Asia rather than East Asia sensu Komarov (1908, 1947) is best thought of as the place of origin, and steppe is treated as the ancestral ecotype, rather than forest as previously suggested, which was exemplified by *C. arborescens*, then regarded as the most primitive species (Sanchir 1979; Zhao 1993; Zhang 1998, 2004, 2005). Substantially, many changes result from the arid ancestral attributes of the genus.

According to Linder (2008), plant species radiations can be divided into so-called old radiations (mature radiations) and recent and rapid radiations. The former were climatically and geologically stable throughout the Neogene, whereas the latter are typical of younger (Pliocene) environments. In *Caragana*, we found that most radiations were mature in the Neogene, and that diversifications at section and series are most Miocene (Zhang and Fritsch 2010). However, recent and rapid radiations certainly are significant, because of the role of further intense aridification from the latter Miocene to Pliocene.

Conclusion

Based on molecular phylogeny, molecular dating, and the biogeography of extant species distribution areas and ecotypes of the genus, the ancestor of *Caragana* is inferred to have had a crown age of ca. 16 Ma in the middle Miocene, and the ancestral attributes of appearing in the Junggar roughly south of Altai-Balkhash Lake, located in the arid steppe belt. The ecotype of the ancestral species is inferred to have been steppe. The evolutionary dynamic of the *Caragana* origin and diversification is speculated to have come from two factors or geological events: Paratethys withdrawal westward and the QTP uplift, especially the significant stages of QTP uplift, namely, the establishment of the southern and central core QTP, probably in late Oligocene to early Miocene, and the later expansion of the QTP by uplift of northern, eastern, and other portions, perhaps in the latter Miocene and Pliocene. All of these are coupled with the Asian interior aridification process. The ecological and geographical direction of adaptive radiation is indicated to be from the Junggar Basin and Central Asia to East Asia, from the arid belt to the humid belt, and from steppe species to forest and/or to desert species. These conclusions of an arid origin and diversification are thus in contradiction to some previous hypotheses based on morphological evolution, such as an East Asian origin with a forest ecotype.

Acknowledgments We are grateful to two anonymous reviewers and editor-in-chief Prof. Marcus Koch for their valuable comments for improving the manuscript. Funding was provided by China National Key Basic Research Program (2012FY111500, 2014CB954201), and Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

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