

Evolutionary response of *Caragana* (Fabaceae) to Qinghai–Tibetan Plateau uplift and Asian interior aridification

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Received: 8 March 2010 / Accepted: 1 July 2010 / Published online: 31 July 2010
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Abstract *Caragana* is endemic to temperate Asia, with most species distributed on the Qinghai–Tibetan Plateau (QTP) and in Northwestern China. Consequently its biogeography should be hypothesized to have been affected by QTP uplift. To examine the biogeography of *Caragana* in relation to QTP uplift and consequent interior aridification, we conducted molecular dating analyses based on three genes (ITS, cpDNA *trnS-trnG* and *rbcL*). Results from relaxed Bayesian BEAST, relaxed Bayesian Multidivtime, and PL (penalized likelihood) indicate that QTP uplift, especially the onset of Himalayan motion at 21–17 Ma, triggered the origin of *Caragana* (with estimated ages 16–14 Ma). The subsequent QTP rapid uplift at 8 Ma is inferred to have driven the evolution and diversification of the three major clades of *Caragana*: section *Caragana* (northern China and the Junggar–Altai–Sayan region), section *Frutescentes* (Central Asia), and sections *Bracteolatae* and *Jubatae*, centered in the QTP. A rapid and active speciation process occurring in the QTP intense uplift at 3.4–1.8 Ma, is indicated by the chronogram.

Keywords *Caragana* · Molecular phylogenetic dating · Biogeography · Qinghai–Tibetan Plateau uplift · Himalayan motion

Introduction

The genus *Caragana* (Fabaceae), comprises approximately 100 species belonging to five sections restricted to temperate Asia (Fig. 1). The major morphological differences in the genus involve the leaflets (either pinnate or palmate) and the rachis (either deciduous or persistent; Moore 1968). The three largest sections are:

- section *Caragana* with pinnate leaflets and a deciduous rachis (temperate forests of eastern Asia or the Altai–Sayan and adjacent regions; Fig. 1);
- section *Bracteolatae* with pinnate-persistent leaflets and a persistent rachis (cold–temperate regions of the QTP (Zhang 1997a); Fig. 1); and
- section *Frutescentes* with palmate leaflets and a persistent rachis (widespread in arid regions of Central Asia (Moore 1968; Zhang 1998); Fig. 1).

These three sections were recovered in a phylogenetic analysis of three genes (ITS, *trnS-trnG* and *rbcL*) with strong support (Zhang et al. 2009a). The variation in distribution patterns and ecological adaptations of these sections in the QTP and Central Asia suggests that *Caragana* can be used to explore evolutionary responses to the QTP uplift and the aridification of the interior of temperate Asia.

On the basis of morphological variation and distribution patterns, Komarov (1908, 1947) used *Caragana* and other four genera to hypothesize floristic connections between China and Mongolia. He suggested that *Caragana* originated in Eastern Asia, perhaps in one part of eastern China.

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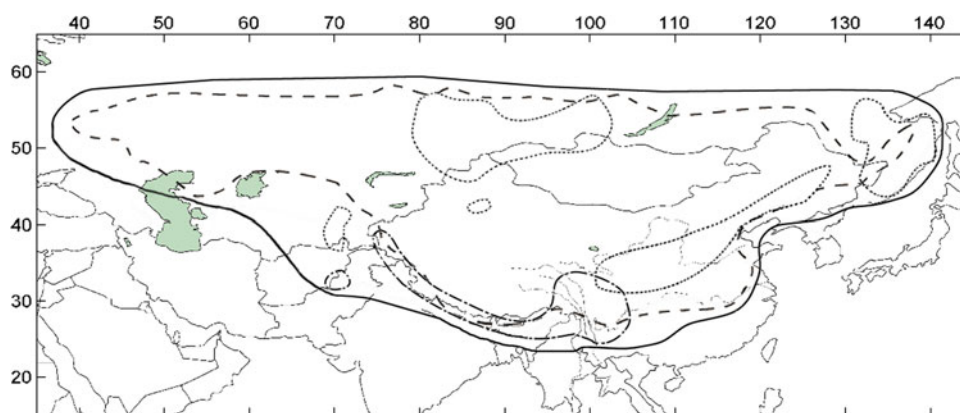
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Fig. 1 Line, distribution of *Caragana*; dotted line, distribution of *Caragana* section *Caragana*; dash-dotted line, distribution of *Caragana* section *Frutescentes*; broken line, distribution of *Caragana* section *Bracteolatae*



Alternatively, Moore (1968) inferred a Central Asian origin, specifically near southern Lake Balkash in the Tian Shan and adjacent Mongolia, where most series of the genus can be found. Sanchir (1979) and Zhao (1993) proposed that *C. arborescens*, with numerous pairs of pinnate leaflets, a deciduous rachis, a diploid chromosome number of $2n = 16$, and a temperate distribution, is the ancestral species of the genus instead of *C. sinica* with a triploid chromosome number of $2n = 24$ proposed by Komarov (1908, 1947). A similar conclusion to that of Sanchir (1979) and Zhao (1993) was reached as a result of analytical approaches such as Component, ancestral area, and dispersal and vicariance analyses (Zhang 1998, 2004, 2005). The time of origin and diversification of *Caragana*, however, was poorly understood because of the lack of fossil and DNA-sequence evidence, and dating approaches for this genus.

Within the last decade, especially, molecular phylogenetic dating techniques have been increasingly employed in systematics and biogeography because they provide a means of examining the temporal origin and evolutionary process of taxa. A molecular phylogenetic analysis based on three genic regions for *Caragana* was recently conducted (Zhang et al. 2009a). Results recovered three strongly supported major clades corresponding to three of the five sections within the genus. Here we examine the historical biogeography of *Caragana* by employing molecular dating approaches based on the phylogenetic estimate of Zhang et al. (2009a) in the context of the major Tertiary geological events that have been inferred to have shaped the evolution of flowering plants in Central Asia. Specifically, *Caragana* can be used to assess the effects of the QTP uplift and consequent interior aridification in cold-temperate Asia.

Materials and methods

Taxon samples and phylogenetic estimate

Taxon samples are the same as those employed in the phylogenetic analysis of Zhang et al. (2009a). These

samples cover all of the sections and series of *Caragana* and all major geographic areas in which the species of the genus occur (Fig. 1).

Three genic region datasets (ITS, *trnS-trnG*, and *rbcL* comprising 3,506 bp in total) were used in the phylogenetic analyses of Zhang et al. (2009a). The resulting maximum likelihood phylogram here is used as input for molecular phylogenetic dating.

Modeltest

The model of DNA substitution that best fit the data was found via the Akaike information criterion (AIC) based on the log likelihood scores of 56 models by using Modeltest 3.5 (Posada and Crandall 1998) and 28 models by using MrModeltest ver. 1.1 (Nylander 2004). The GTR+I+G model was selected by both Modeltest and MrModeltest as the best model. The values yielded by Modeltest and MrModeltest, i.e. the nucleotide frequencies $A = 0.3032$, $C = 0.1755$, $G = 0.2086$, $T = 0.3128$, substitution rate data $R_{ac} = 1.4351$, $R_{ag} = 1.9771$, $R_{at} = 0.5602$, $R_{cg} = 0.7200$, $R_{ct} = 2.8738$, and $R_{gt} = 1.0000$, the gamma shape parameter 0.9119, and an assumed proportion of invariable sites 0.5884, were used as input for the BEAST analysis (see below).

Dating

Fossil calibration

Although there are no fossil records of *Caragana* and relatives, for example the Hedysaroid and Astragaleae clades (Lewis et al. 2005; Lock 2005), there is a rich fossil record for Fabaceae. Using many of Fabaceae fossils as constraints, Lavin et al. (2005) and Wojciechowski (2005) estimated the time of origin of the inverted-repeat-lacking clade (IRLC), the Astragalean clade (ca. 39 Ma), and the Vicioid clade (36 Ma). Lavin et al. (2005) estimated the age of the crown node of the clade comprising *Caragana*

and *Astragalus* as ca. 33 Ma. Lavin et al. (2005) and Wojciechowski (2005) estimated the age of the Hedysaroid clade comprising *Caragana* and *Hedysarum* as ca. 29.3 and 21.4 Ma, respectively. These indirect but credible estimates are consequently employed into this study, because of the lack of fossils of *Caragana*, *Hedysarum*, and *Astragalus*. Because only one species each of *Caragana* (*C. arborescens*) and *Astragalus* were used in the analyses mentioned (Lavin et al. 2005; Wojciechowski 2005), a conservative and reduced fixed root age representing the divergence time between *Caragana* and *Astragalus* should be decided as ca. 28 Ma, and that between *Hedysarum* and *Caragana* should be decided as ca. 21 Ma.

Dating implementation

Phylogenetic dating was conducted by three approaches: relaxed Bayesian uncorrelation rate (BEAST), relaxed Bayesian autocorrelation rate (Multidivtime), and penalized likelihood (r8s).

BEAST v1.46 (<http://www.beast.bio.ed.ac.uk/>) was used to estimate divergence times (Drummond and Rambaut 2007). Best-fit models of nucleotide substitution GTR+I+ Γ and Γ distribution as site heterogeneity model, and parameters for the priors in BEAST were provided by Modeltest. A Yule process speciation and UPGMA were used to generate a starting tree, and an uncorrelated log-normal model of rate variation was selected as priors. A normal distribution was chosen. Tracer v1.4 (Drummond and Rambaut 2007) was used to measure the efficient sample size of each parameter and mean and 95% confidential intervals. Two repeated MCMC analyses were run for 20,000,000 generations (burnin 10%) with sample size every 1,000 steps. The mean and 95% confidential intervals of nodes were assessed by TreeAnnotator v1.4.8. and FigTree v.1.2.4 (Drummond and Rambaut 2007).

In r8s version 1.7 (<http://www.ginger.ucdavis.edu/r8s/>) the penalized likelihood (PL; semi-parametric rate smoothing; Sanderson 2002) method and TN algorithm were used. ITS + cpDNA maximum likelihood tree (Zhang et al. 2009a) was used as a base of r8s. The crown age 21 Ma of *Hedysarum* and *Caragana* was used as the calibration age. The smoothing parameter of the cross-validation option in r8s was estimated (smoothing = 1) for PL. The outgroup *Astragalus* was pruned.

Multidivtime consists of PAML (<http://www.abacus.gene.ucl.ac.uk/software/paml.html>) and Multidivtime (<http://www.statgen.ncsu.edu/thorne/multidivtime.html>). Four programs, baseml, Estbranches, paml2modelinf, and Multidivtime, were implemented. Baseml estimated the terms of the substitution model. Estbranches estimated branch length and a variance–covariance matrix under the F84 model and

Γ distribution. Multidivtime estimated rates and times with the Bayesian MCMC method. A Bayesian MCMC analysis was run for 10,000,000 generations, sampled every 100 generations, burn in 100,000, rttm = 0.21, rttmsd = 0.021. The implementation of the four programs followed a step-by-step procedure available at <http://www.plant.ch/software.html>.

Results

The phylogenetic dating results of the BEAST, Multidivtime, and r8s analyses are summarized in Table 1, which focuses on six estimated ages of important clades at the sectional level.

The two relaxed molecular clock approaches, BEAST and Multidivtime, treat rate variation among phylogenetic tree branches as uncorrelated and autocorrelated, respectively. Multidivtime requires a phylogenetic tree topology as input, but BEAST does not. Both are run under a Bayesian MCMC algorithm (Renner 2005; Welch and Bromham 2005). Generally, divergence times estimated from these methods are congruent, but those from Multidivtime are generally higher than those of BEAST (e.g. Zhang et al. 2008). This is also true of our results.

The estimated divergence times by using the three dating approaches of the major clades of *Caragana* differ from each other. It is common for estimates to differ, for example, among the three dating approaches (Renner et al. 2008), between r8s and Multidivtime (Knapp et al. 2005), and between BEAST and r8s (Nie et al. 2008). Recently, BEAST is in favor with many people (e.g. Verboom et al. 2009; Night and Reed 2009; Wilson et al. 2009; Cowman et al. 2009). Additionally, because ingroup calibration points are important for dating the nodes of the ingroup, our lack of congruence among the three approaches is likely to result from the lack of calibration points within *Caragana*. Thus, the BEAST dating chronogram is presented (Fig. 2) and the following discussion will mainly rely on its results.

Discussion

From our results we infer that the origin of *Caragana* dates from Miocene. Based on sediments, crust, vegetation, palynology, and macrofossils of the Loess Plateau in eastern QTP, etc., Shi et al. (1998, 1999) and Li and Fang (1998) assumed three phases of the QTP uplift. The first phase (Gangdese motion ca. 40, 45–38 Ma) is characterized by the Indian plate subducting under the Eurasian plate, resulting in the rise of the Gangdese Mountains. The second phase (Himalayan motion ca. 21, 25–17 Ma) is characterized by the rise of the QTP to ca. 2,000 m, the

Table 1 Phylogenetic dating results for *Caragana* and five relatives using BEAST, Multidivtime, and PL-r8s

		BEAST	Multidivtime	PL r8s
<i>Astragalus</i> + <i>Hedysarum</i> + <i>Caragana</i> 28, <i>Hedysarum</i> + <i>Caragana</i> 21 (constrained)				
1	<i>Caragana</i>	16.15 (9.96–20.34)	14.96 (11.16–18.22)	13.52
2	Sect. <i>Caragana</i>	7.99 (4.08–12.62)	8.23 (4.04–13.22)	6.18
3	Sect. <i>Frutescentes</i>	7.49 (2.52–12.63)	10.45 (1.00–14.55)	3.55
4	Sect. <i>Bracteolatae</i>	4.45 (0.75–8.54)	6.00 (3.47–10.64)	3.02
5	Sect. <i>Jubatae</i> + <i>Bracteolatae</i>	7.63 (1.22–14.3)	9.82 (6.15–13.85)	4.34
6	Sect. <i>Jubatae</i> + <i>Spinosae</i>	5.46 (1.00–15.08)	4.14 (0.99–8.84)	4.85

In BEAST and Multidivtime, the mean and 95% confidence intervals of the nodes are shown. The sectional classification of *Caragana* follows Zhang (1997b) and Zhang et al. (2009a)

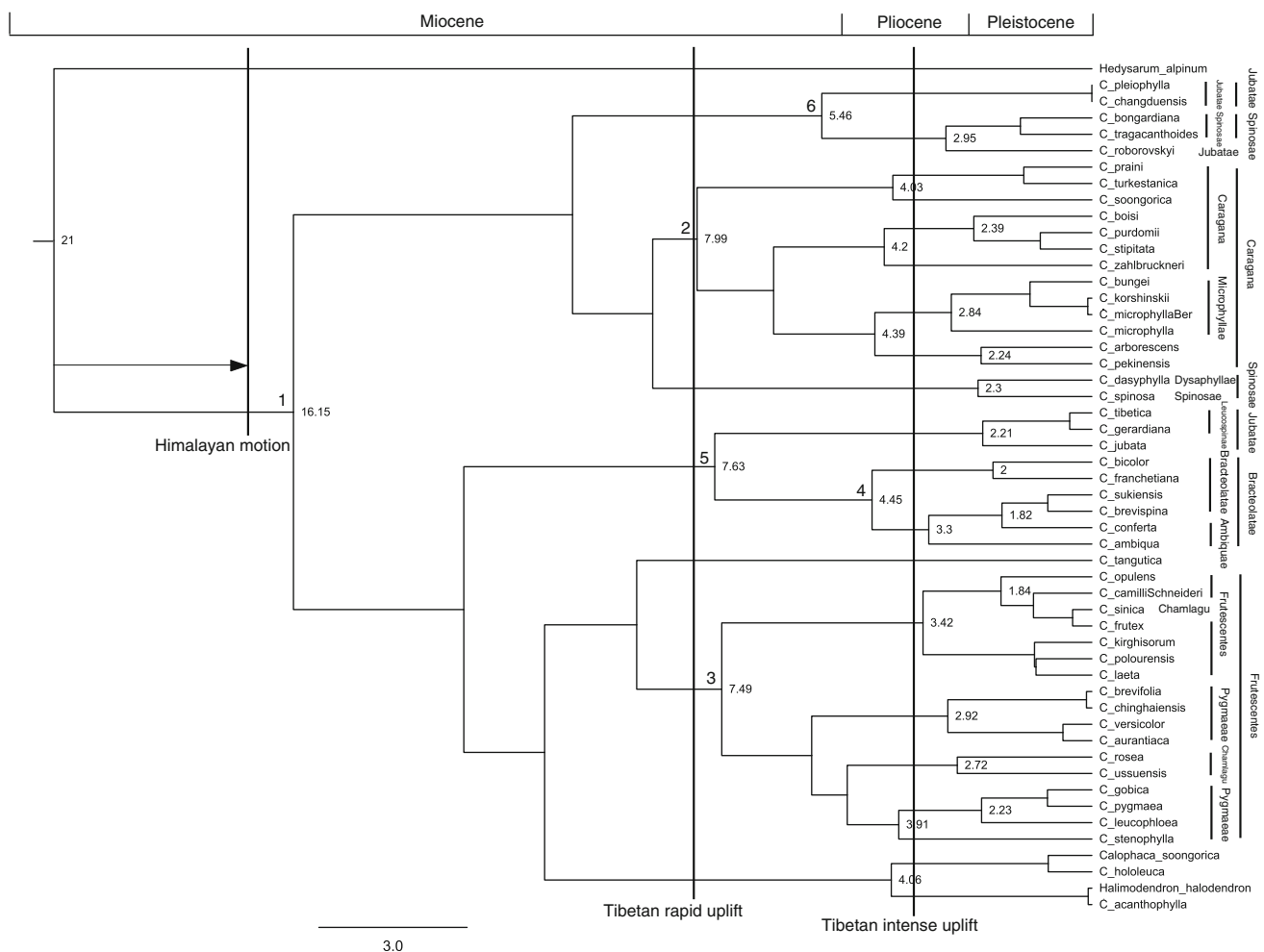


Fig. 2 Chronogram of divergence times within *Caragana* based on relaxed Bayesian analysis in BEAST. The tree is that from a combined data set from the genic regions ITS, *trnS-trnG*, and *rbcL*

westward withdrawal of the Paratethys Sea, the aridification of interior Asia (Guo et al. 2002), and the onset of the Asian monsoon (An et al. 2001). The third phase (QTP intense uplift ca. 3.6 Ma) is accompanied by the formation of the modern Asian monsoon. Another important event is suggested by Harrison et al. (1992) and known as the rapid

QTP uplift at 8 Ma. The following discussion will be based on these chronographic scales. We will link the estimated ages of *Caragana* from the phylogenetic dating analyses with these geological events to explore the speciation processes that are assumed to have given rise to the genus and its major clades.

Himalayan motion

Himalayan motion 20 (21–17) Ma triggered the birth of Caragana

Harrison et al. (1992) and Molnar et al. (1993) provided evidence of a rapid uplift at 21–18 Ma in southern Tibet and Himalaya. Shi et al. (1999) inferred that the second uplift of Tibet occurred in the early Miocene 25–17 Ma. This uplift raised the mean altitude of the QTP to 2,000 m and was termed the Himalayan motion. Because the Himalayan motion is one of the most significant events in the overall uplift of the QTP, *Caragana*, with estimated age at 16–14 Ma, closely follows this geological event 21–17 Ma; the origin of *Caragana* can, therefore, be linked temporally to the Himalayan motion.

Many plant and animal groups for which molecular phylogeny and biogeography have been investigated, have evolved in response to the early Miocene QTP uplift. For example, *Nannoglottis* (Asteraceae) (Liu et al. 2002), comprising ca. eight species endemic to Tibet, reached Tibet from the Southern Hemisphere in the Oligocene–Eocene and then re-diversified with the uplift of the plateau. The biogeography of *Nannoglottis* coincides with the known geological and palaeobotanical history of the QTP. The explosive radiation of the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae) (Liu et al. 2006) also endemic to the QTP, with an estimated age of 20 Ma, occurred during the major uplifts of QTP from the early Miocene to the Pleistocene. The time of diversification of Chinese sisorid catfish centered in QTP was estimated as at the Oligocene–Miocene boundary (19–24 Ma), and was related to three uplift events of the QTP (Guo et al. 2005).

Wang et al. (2004) speculated that the large-scale colonization of cushion-like *Androsace* (Primulaceae) in the QTP related to the QTP uplift since the Miocene, while another phylogenetic origin of this group in Europe probably related to climate oscillation and glacial shuttlecock at the boundary of Tertiary and Quaternary. Chen et al. (2005) presented molecular dating suggesting that separation of *Crawfordia*, *Metagentiana*, and *Tripterosperrum* from *Gentiana* (Gentianaceae) is likely to have occurred about 21.4–11.4 Ma. The speciation and rapid radiation of these three genera is likely to have occurred in western China as a result of upthrust of the Himalayas during the late Miocene and the Pleistocene. Wang et al. (2009a) inferred that the diversification and island-like radiation of *Saussurea* (Asteraceae) in the QTP was during 14–7 Ma. Divergence times of three genera of *Dolomiaea*, *Diplazoptilon*, and *Xanthopappus* (Asteraceae) endemic to the Himalayas, indicated that *Dolomiaea–Frolovia* probably occurred 13.6–12.2 Ma, the divergence time of *Diplazoptilon* is around 5.7–4.7 Ma, and that of *Xanthopappus* is

around 2.0–1.6 Ma (Wang et al. 2007). *Caragana*, with an estimated birth age 16–14 Ma, can be regarded as another case of a clade originating as a response to the Himalayan motion of the QTP uplift.

Inferring ancestral attributes of Caragana

Komarov (1908, 1947) considered *Caragana sinica* of Eastern Asia the ancestral species in *Caragana*, and therefore eastern Asia as the area of origin of the genus. This view was rejected by Moore (1968) who showed that *C. sinica* is triploid and therefore not primitive, and Moore (1968) made the alternative suggestion that Central Asia, specifically southern Lake Balkash in the Tian Shan adjacent to Mongolia, was the area of origin because most sections and series of the genus occur there. Sanchir (1979), Zhao (1993) and Zhang (1997b, 2004) proposed that *C. arborescens*, with numerous pairs of pinnate leaflets, a deciduous rachis, a diploid chromosome number, and a temperate distribution, is the ancestral species.

Both *C. arborescens* and *C. sinica* are relatively young according to our chronogram, with estimated ages of 2.24 Ma (divergence between *C. arborescens* and *C. pekinensis*) and 0.39 Ma (divergence between *C. sinica* and *C. frutex*). Therefore, these hypotheses are not supported by our data. The presence of an arid vegetation zone throughout Central China in what is now the center of diversity of *Caragana* during the early Tertiary or Late Cretaceous–Paleogene (Shi et al. 1998; Tao 1992; Guo et al. 2008) might suggest that the habitat condition to which *Caragana* first adapted was arid. In the middle Miocene, ca. 14–16 Ma, however, at the inferred time of origin of *Caragana*, the arid and grassland zone in China was withdrawing westward while the humid and forest zone was expanding northwestward from southern and eastern China (Shi et al. 1998; Tao 1992). Therefore, this climate and vegetation event suggests that the origin of *Caragana* was affected most by humid climate and forest vegetation from the eastern distribution area. Consequently, a balanced opinion for inferring the ancestral adaptation regime of *Caragana* perhaps should be that it has attributes of both arid and mesic–temperate climate: species in the western part of the distribution area in *Caragana* seems to possess arid adaptations whereas those of the east possess mesic–temperate adaptations.

QTP uplift ca. 8 Ma drove the diversification of the three major clades within Caragana

On the basis of the molecular phylogeny (Zhang et al. 2009a), three main groups were identified in *Caragana*, i.e. the pinnate group, palmate group, and spiny group. These groups roughly correspond to the phylogenetic results

based on morphological characters—leaflet pinnate or palmate, leaf rachis deciduous or persistent (spiny) (Moore 1968; Zhang 1997b). The three groups are: the pinnate group (section *Caragana*), which mainly has pinnate leaflets and a deciduous leaf rachis; the palmate group (section *Frutescentes*), which mainly has palmate leaflets and a persistent leaf rachis (spiny), and the spiny group (sections *Bracteolatae* and *Jubatae*), which mainly has pinnate leaflets and a persistent leaf rachis (spiny; Zhang et al. 2009a). The pinnate group occurs mainly in Eastern Asia and the Altai–Sayan–Junggar region, the palmate group occurs mainly in Central Asia and the Mongolian Plateau, and the spiny group occurs mainly in the QTP (see Fig. 1). These three groups have estimated ages of 7.99, 7.49, and 7.63 Ma, respectively.

QTP rapid uplift ca. 8 Ma

On the basis of thermochronologic, sedimentologic, oceanographic, and paleoclimatic studies, Harrison et al. (1992) suggested 8 Ma as a time of rapid QTP uplift. This has been subsequently supported by many studies (e.g. Molnar et al. 1993). This uplift may be the trigger of the origin of sections *Bracteolatae* and *Jubatae* pro parte. The spiny character of the groups should be thought as an adaptation to the cold climate (Zhang 1997a) that developed as a result of the rapid uplift of the QTP.

It is reasonable to infer that the QTP rapid uplift at 8 Ma triggered the divergence and formation of all three groups in terms of three estimated time scales within *Caragana*. This is similar to plant and animal examples with phylogenetic origin, diversification, and radiation around 8 Ma, for example the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae) (Liu et al. 2006), *Rheum* (Polyganaceae) (Wang et al. 2005), *Androsace* (Primulaceae) (Wang et al. 2004) and *Saussurea* (Asteraceae) (Wang et al. 2009a), and the glyptosternoid catfishes (Peng et al. 2006). Furthermore, as one of the consequent effects of the rapid uplift should be climate change, we explain the divergence and formation of three groups from the resulting climate status in the following sections.

Cold climate ca. 8 Ma and spiny group

Cerling et al. (1993, 1997) proposed that there was a global expansion of C_4 biomass at 8–6 Ma resulting from the evolution of C_4 photosynthetic grasses, which was accompanied by a cooling climate and increasing aridity. Recently, those effects of climate change and low CO_2 are speculated to be the driving force of the origin of C_4 photosynthetic grasses (e.g. Vicentini et al. 2008; Christin et al. 2008; Bouchenak-Khelladi et al. 2009). Shi et al. (1999) considered that cooling and the arid climate began

8.5 Ma. In the QTP and adjacent areas, this cooling and aridity is likely to have resulted from the QTP uplift. Consequently, it is likely to have led to the origin and divergence of cold-adapted and arid-adapted groups of organisms. The clade comprising sections *Bracteolatae* and *Jubatae* pro parte, inferred to have originated at 7.63 Ma in the QTP, is consequently likely to have arisen in response to cooling climate. The typically persistent rachis and spiny character, found only in the QTP, can be regarded as a consequence of the cooling climate.

Arid climate ca. 8–8.55 Ma and palmate group

After uplift resulting from the Himalayan motion, a large arid zone occupied in central China in the Paleogene and withdrew northwestward during the Neogene (Shi et al. 1998; Tao 1992; Guo et al. 2008). This event is suggested to have appeared between ca. 8–8.55 Ma and was based on, for example, the palynology of stratum and animal fossils in the Linxia Basin area in the northeastern QTP. It was shown that at ca. 8.55 Ma the vegetation changed from warm–humid forest vegetation which existed in northwestern China from 21.8–8.5 Ma to arid grassland (Shi et al. 1998). The increasing extent of the eastern summer wind from the Pacific and Indian Ocean and the northwestern winter wind ended at ca. 8.5 Ma. Wind-blown sediments from the QTP accumulated over a wide area in the Loess Plateau ca. 8 Ma (An et al. 2001; Zheng et al. 2000). Northern Pakistan became arid at ca. 8 Ma (Quade et al. 1989), North America and South America were also arid at ca. 8 Ma (Quade and Cerling 1995). This indicates global aridity was beginning at ca. 8–8.55 Ma. The arid palmate group section *Frutescentes*, with an estimated age of 7.49 Ma and occurring in Central Asia and especially northwestern China, just coincides with this background of geological, climatic, and vegetation change in time and space. The rapid QTP uplift, Asian interior aridification, and vegetation change are inferred to have spurred speciation and evolution in this group. Therefore, the rapid QTP uplift not only drove the divergence of the cold spiny group in the QTP, but also the arid-adapted palmate group widespread in Central Asia.

Warm climate ca. 8 Ma and pinnate group

An et al. (2001) summarized three stages of Asian climate resulting from Tibet uplift. Of these, the one at 9–8 Ma is the most significant for its enhanced aridity in the Asian interior and the onset of the Indian and Eastern Asian monsoons. The winter-enhanced cold and arid climate can be interpreted to have affected the xerophytic group section *Frutescentes* 7.49 Ma mainly in Central Asia mentioned above, whereas the summer monsoon-enhanced warm and

humid climate can be interpreted to have driven the formation of the mesophytic group section *Caragana* 7.99 Ma in northern China and northeastern China–Far East. The mesophytic groups in the Altai–Sayan–Junggar region and other areas (Fig. 1) can be explained as the origin of the temperate forests, and its adjacent area distribution could be interpreted as radiation from these forests. From the perspectives of climate ca. 8 Ma, the spiny group in the QTP probably resulted largely from a cold and arid climate, the palmate group mainly from Asian interior aridity, and the pinnate group in northern China and northeastern China–Far East mainly from the warm and humid climate of the summer monsoon. All of these climates resulted from the rapid QTP uplift.

QTP intense uplift ca. 3.6 Ma driven a rapid and active speciation ca. 3.4–1.8 Ma

Although there are different opinions on the timing of the QTP intense uplift, either at 8 Ma (Harrison et al. 1992; Molnar et al. 1993), 14–15 Ma (Coleman and Hodges 1995; Spicer et al. 2003), or late 3.6 Ma (Li and Fang 1998; Zhong and Ding 1996; Shi et al. 1998, 1999; Zheng et al. 2000), 3.6 Ma is in any case an important scale. Furthermore, Li and Fang (1998) and Shi et al. (1999) proposed three stages of the QTP intense uplift, i.e. the Qingzang motion 3.6–1.7 Ma, the Kunhuang motion 1.1–0.6 Ma, and the Gonghe motion 0.15–present. As the plant responses of this geological event, Chen et al. (2005) reported that the current species of *Crawfordia*, *Metagentiana*, and *Tripterospermum* (Gentianaceae), which grow in coniferous forest, alpine shrub, and alpine regions, have an estimated range of 6.2–0.4 Ma. As mentioned above, the divergence of the “alpine shrub” versus the “coniferous forest” clades within *Nannoglottis* was estimated at ca. 3.4 Ma when the QTP was intensely uplifted and the onset of the development of coniferous vegetation began (Liu et al. 2002). The diversification of *Gentiana* section *Cruciata* (Gentianaceae) at ca. 4 Ma confirmed the long-standing hypothesis that the alpine plants in Central Asia and Europe originated from the QTP and/or West China (Zhang et al. 2009b). A phylogeography of *Aconitumgymmandrum* (Ranunculaceae) (Wang et al. 2009b), like other groups endemic to QTP (Qu et al. 2005; Zhang et al. 2005; Meng et al. 2007; Chen et al. 2008), shows that the alpine plants endemic to the QTP had independent glacial refugia to have existed during the last glacial maximum (LGM). Therefore, 3.6 Ma of the intense uplift is accepted as a basis for discussion.

Phylogenetic dating of *Caragana* at ages 3.4–1.8 Ma consists of a total of 14 nodes (Fig. 2), illustrating rapid and active speciation during this period that falls into the range of the Qingzang motion at ca. 3.6–1.7 Ma. Typically, divergences appear in different areas and taxa: Junggar

2.95 Ma (*C. bongardiana*, *C. tragacanthoides*, *C. soongorica*), northern China 2.39 Ma (*C. purdomii*, *C. stipitata*, *C. boisi* to the east of Sichuan), northern China and eastern Mongolia series *Microphyllae* 2.84 Ma (*C. bungei*, *C. microphylla*, *C. korshinskii*), section *Spinosae* 2.3 Ma (*C. dasyphylla*, *C. spinosa*), Hengduan Mountain 2 Ma (*C. bicolor*, *C. franchetiana*), along the Himalaya (*C. sukiensis*, ..., *C. ambigua*), the eastern Asian series *Chamlagu* 2.72 Ma (*C. rosea*, *C. ussuriensis*), and the Central Asian series *Pygmaeae* (*C. gobica*, ..., *C. stenophylla*), etc. This shows that the Qingzang motion 3.6–1.7 Ma of the QTP intense uplift probably drove a rapid and active series of speciation events in *Caragana* 3.4–1.8 Ma. These divergence times are generally found at the series level within *Caragana*.

In short, with a series of morphological variation and arid and cold ecological adaptation of leaflet pinnate or palmate, leaf rachis deciduous or persistent (spiny), and an obvious phylogenetic diversification of three groups (Zhang et al. 2009a), *Caragana* has its leaved genetic signature and evolutionary response to step-forward uplifts of the QTP, particularly Himalayan motion 20 Ma, cooling and arid climate 8 Ma, and intense uplift 3.6 Ma (sensu Shi et al. 1998, 1999; Li and Fang, 1998). In other words, these striking diversifications within *Caragana* were driven by the QTP uplift and Asian interior aridification. This fit coincidence between the *Caragana* evolutionary process and the QTP uplifts since the Miocene is different from other studied groups in the QTP. Of course, *Caragana* and other biological cases are, unquestionably, all evidence of the geological hypotheses of the QTP uplift and Asian interior aridification.

Acknowledgments Thanks to Peng Zhang (University of California, Berkeley, USA) for molecular dating help, Jianquan Liu (Lanzhou University, China) for discussion of the QTP biogeography, and to an anonymous reviewer for a very useful suggestion on QTP plant biogeography. Funding is from the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, and the Chinese Academy of Sciences (KSCX2-YW-R-136, KSCX2-YW-2-069).

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