A preliminary molecular historical biogeography of *Caragana* (Leguminosae) based on ITS and *trn*L-F DNA sequence data

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Abstract: The nrDNA internal transcribed spacer (ITS) and cpDNA *trn*L-F internal spacer (IGS) sequence data of *Caragana* eight species and one outgroup *Halimodendron halodendron,* was employed to reconstruct a phylogenetic tree, then the area relationship was analyzed by means of component analysis (CA), Brooks parsimony analysis (BPA), and dispersal-vicariance analysis (DIVA), six areas were selected from two divided distributions of East Asia and Tethys in *Caragana*. The phylogenetic tree indicated that there were three distinctive groups, which were attributed to some morphological characters, first with pinnate foliage and deciduous rachis, second with palmate foliage and persistent sclerotic stick rachis, and third with pinnate foliage and persistent sclerotic stick rachis. The results of CA and BPA illustrated general area relationships. An explicit area relationship should be Altai—Sayan, Far East—NE China and North China (Hengduan Mountains). DIVA recognized several explanatory vicariance and dispersal events. As the scenario of *Caragana* distribution pattern, it looks like the vicariance versus dispersal plays more important role. In vicaraince, there are not only the isolated far-distance vicariance, but also the adjacent vicariance especially a vicariance between Hengduan Mountains and North China.

Keywords: *Caragana*, ITS, *trn*L-F, phylogeny, cladistic biogeography, DIVA, distribution pattern

1 Introduction

Caragana Fabr., belonging to Papilionoideae and Leguminosae (Polhill, 1981), has about 70 species and mainly occurs in the temperate Asian region, China, Mongolia, Central Asia and adjacent region. In generally, this genus was considered as a drought shrub group because some species could form the community in drought steppe to desert and protect water and soil.

Because of large morphological variation and interesting distribution pattern, the generic classification and phylogeny have studied by many taxonomists (Pojarkova, 1945; Moore, 1968; Sanchir, 1980, 1999; Gorbanova, 1984; Liu, 1993; Zhao, 1993; Zhang, 1997b) since Komarov's (1908) first monograph of the genus. Even if large morphological differentiation in this genus, the classifications are still not identical so far. On phylogeny, experimental evidences have been accumulated from chromosome karyotype and pollen morphology (Moore, 1968; Zhang et al., 1997; Zhou et al., 2002). Recently we obtained the ITS DNA sequence data of 28 species belonging to 5 sections in the genus, and used them to reconstruct phylogenetic tree. As a legume group, there is no sequence data so far focus on *Caragana*, only a few species such as *C. arborescens* and *C. sinica* were concerned in legume molecular systematics (Sanderson and Liston, 1995; Sanderson and Wojciechowski, 1996; Wojciechowski et al., 1999).

From the former studies (Zhang, 1998, 2004; Zhang et al., 2002), we found some interesting distribution patterns in the genus. For instance, 13 areas in

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Caragana were determined, then they were divided into East Asian and Tethys group (Zhang, 1998), *C. arborescens* occurs in three areas, Far East-Northeast China, Altai-Sayan and North China. This distribution of *C. arborescens* was explained as the vicariance among three areas (Zhang, 1998, 2004). In those narrative vicariances in *Caragana*, some happen among isolated areas, most are between adjacent areas. The latter was described as short dispersal (Zhang, 1998, 2004). These distributions need further analytical study. However, within Asia especially China, there is hitherto a few papers in detail concerning vicariance distribution pattern and using these typical analytical biogeographical approaches (Humphries and Parenti, 1999). Using these approaches, most papers hitherto dealt with biogeography on the large range, the global, the intercontinental disjunction, the northern temperate, the East Asia and North America disjunction (Donoghue et al., 2001; Wen, 2001; Xiang and Soltis, 2001; Renner, 2004). According to the floristic division and vegetation types, *Caragana* as a temperate Asian distribution, could be regarded as 13 areas of obvious differentiation (Fig. 1) (Zhang, 1998). Therefore, we want to attempt to discuss distribution pattern at a small region and focus on some interesting distribution events, such as vicariance and dispersal (Xiang and Soltis, 2001; Jønsson and Fjeldsa, 2006; Spalik and Downie, 2006). On the other hand, phylogenetic tree should been reconstructed using DNA sequence data instead of our former morphology. Thus, the goals of the present paper are: (1) the related areas are extracted from our previous division of East Asian and Tethys so that the temperate Asian biogeography especially within China, where with some vicariance and dispersal events, can be pay attention to the genetic distribution; (2) a molecular phylogenetic tree should been reconstructed as a base; (3) using typical analytical biogeography based on molecular phylogeny to analyze the distribution pattern, then test our previous related phylogenetic and biogeographical hypotheses.

2 Materials and methods

2.1 Areas and taxa sampling

Based on the morphological taxonomy of *Caragana* and its related taxa (Polhill, 1981), *Caragana* is regarded as a good genus and never confused with other taxa in legumes. Therefore, *Caragana* is treated as a monophyletic group for cladistics and phylogeny (Zhang, 1997b; Zhang et al., 2009). *Halimodendron halodendron* (Pall.) Voss, the most related taxon to *Caragana*, was regarded as the outgroup.

In order to focus on the temperate Asian distribution pattern in *Caragana*, eight species belonging to three sections and five series, were selected (Table 1). In *Caragana*, Sect. *Caragana* and Sect. *Frutescen-*

Table 1 Material of eight species in *Caragana* and *Halimodendron halodendron*, the classification of *Caragana* follows Zhang (1997)

Taxa	Collectors	Sources	GenBank accession number	
			ITS	*trn*L-F
Sect. *Caragana*				
Ser. *Caragana*				
C. arborescens Lam	Zhang M L 00-201	Xinjiang, Altai	GQ338263	GQ890296
C. boisii Schneid.	Zhang M L, Kang Y 00-121	Sichuan, Lixian	GQ338266	GQ890298
C. stipitata Kom.	Kang Y 00-55	Shaanxi, Qingling	GQ338286	GQ890303
Ser. Microphyllae (Kom.) Pojark.				
C. pekinensis Kom.	Zhang M L 99-56	Beijing, Xiangshan	GQ338278	GQ890300
Sect. *Bracteolatae* M. L. Zhang				
Ser. *Bracteolatae*				
C. bicolor Kom.	Zhang M L, Kang Y 99-178	Sichuan, Markang	GQ338265	GQ890297
Sect. *Frutescentes* (Kom.) Sancz.				
Ser. *Chamlagu* Pojark.				
C. sinica (Buc'hoz) Rehd.	Zhang M L 99-49	Beijing, Xiangshan	GQ338283	GQ890302
Ser. *Frutescentes*				
C. rosea Turcz. ex Maxim	Zhang M L 99-45	Beijing, Beihuashan	GQ338282	GQ890301
C. frutex (Linn.) C. Koch.	Zhang M L 99-258	Xinjiang, Urumqi Bot Gard	GQ338271	GQ890299
Halimodendron halodendron (Pall.) Voss.	Zhang M L 00-279	Xinjiang, Urumqi Bot Gard	FJ537289	GQ890304

tes are monophyletic resolved respectively, another one are morphological intermediate basing on ITS sequence.

These eight species should have a possibility to representative the taxon relationships on high taxonomical level for the genus as shown in Table 1. In addition, *C. bicolor* is endemic to Hengduan Mountains, and also a type species from Sect. *Brateolatae* since it with several related species in this section (Zhang, 1997a). *C. arborescens*, occur in three areas mentioned above; *C. arborescens* and *C. boisii*, a pair of related species and occurring respectively in North China and Hengduan Mountains, were also postulated as a vicariance (Zhang, 1997a).

According to *Caragana* distribution, 13 areas were classified into two large area groups, East Asian group and Tethys group (Zhang, 1998), and six areas were extracted from them (Fig. 1). Similar to taxa selected, two large area groups could also represented by the six areas (Fig. 1) since FE, NC, HD and EA are belong to East Asian group, AS and ES are belong to Tethys group.

Eight species from *Caragana* and *Halimodendron halodendron* as the outgroup, were sequenced in this paper.

2.2 DNA isolation, PCR amplification and sequencing

DNA samples were obtained from fresh, silica-gel-dried leaf tissue following the CTAB method of Doyle and Doyle (1987).

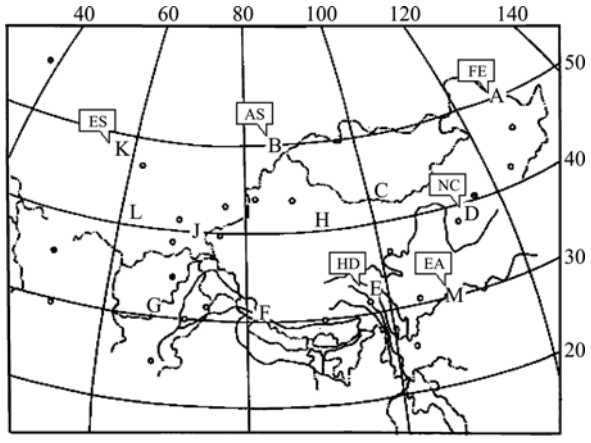

Fig. 1 13 areas were used to treat *Caragana* distribution (Zhang, 1998). Of them six areas were chosen in this paper
A: Far East-Northeast China; B: Altai-Sayan; C: Mongolia Plateau; D: North China -Qinling Mts; E: Hengduan Mts; F: Himalayas; G: Balut-Afghanica; H: Central Asia; I: Tianshan Mts; J: Pamir-Alai; K: Europe-West Siberia; L: Turan; M: East Asia Subtropical.

For ITS, primers “ITS1” and “ITS4” were used in PCR amplification. The sequence of primer “ITS1” was 5’ AGA AGT CGT AAC AAG GTT TCC GTA GC 3’, which was a conservative sequence of the upstream region of rice 17S rDNA. The sequence of “ITS4” was 5’ TCC TCC GCT TAT TGA TAT GC 3’(White et al., 1990). ITS amplification reaction protocol was referring to Kang et al. (2003). For *trn* L-F, primers “c” and “f” were used for amplifying the *trn*L intronm *trn*L 3’ exon, and *trn*L-F intergenic spacer (Tablerlet, et al., 1991). *trn*L-F amplification reaction protocol was following Wang et al. (2002).

PCR products were purified with Pharmacia Biotech aFXtmper DNA and Get Band purification Kit. Sequencing was by using ABI 377 automatic apparatus (PE Company). Primers were the same as that of initial PCR and used singly in forward and reverse reactions. Each base position was examined for agreement between complementary strands.

Sequences were automatically aligned using CLUSTAL W (Thompson, et al., 1994), and a few complemented by hand. All new ITS and *trn*L-F sequences were submitted to GenBank (Table 1). The alignment is available on request from author.

These experiments were yielded at the Systematic and Evolutionary Botany Lab., Institute of Botany, CAS, Beijing.

2.3 Phylogenetic analysis

Phylogenetic tree was reconstructed by using the maximum parsimony and software PAUP 4.0 (Swofford, 1998). Heuristic search option included 100 random addition replicates (exhaustive option and band and branch option were also used), tree bisection reconnection branch swapping, and steepest descent. It was employed to find most equal parsimonious tree. Then strict consensus tree was computed, and bootstrap value was used as the support test. Some tree indices were provided, such as CI, and RI.

2.4 Biogeographical analysis

2.4.1 Component analysis

Basing on the phylogenetic tree and the areas (Fig. 2), the assumption 0, 1 and 2 (Nelson and Platnick, 1981; Zandee and Roos, 1987; Humphries and Parenti, 1999) and Component 1.0 (Page, 1989) were employed to construct the area cladogram to show area relationship.

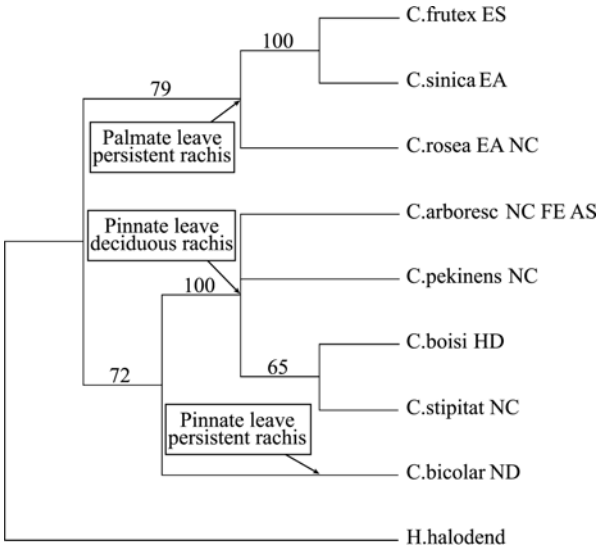

Fig. 2 Phylogenetic tree was reconstructed based on ITS and *trn*L-F sequence data and PAUP program. The outgroup was *Halimodendron halodendron* (Pall.) Voss. Just one parsimonious tree was obtained using heuristic option and bootstrap values on the nodes, length=114, CI=0.9561, RI=0.9206. Three distinctive groups (sections, Table 1) with pane and arrowhead, were illustrated on the tree. And species distributions were also labeled. Areas EA：M, ES：K, NC：D, HD：E, AS：B, and FE：A are infer to Figure 1.

2.4.2 Brooks parsimony analysis (BPA)

Based on the phylogenetic tree and the areas (Fig. 2), the taxa and nodes on the tree were labeled (Fig. 4) (Wiley, 1988; Brooks, 1990; Brooks and McLennan, 2001). Then a coding data matrix was obtained (Table 2) on the basis of the labeled information. Hennig 86 (Farris, 1988) was employed to analyze area relationship.

Table 2 Data matrix used to BPA, areas A-F and taxa 1-8 refer to Figure 4.

Area	Taxa	
A	1	10000000110001
B	2, 3	01100000110002
C	3, 4, 5, 7	00111010011334
D	4	00010000000111
E	4	00010000000111
F	6, 8	00000101001122

2.4.3 Dispersal and vicariance analysis (DIVA)

DIVA 1.1 program (Ronquist, 1996, 1997) was used to treat the information from phylogenetic tree (Fig. 2). At first, the phylogenetic tree was necessary to divide into three trees since there is a paraphyletic group with *C. arborescens*, *C. pekinensis* and two species (*C. boisii*, *C. stipitata*) on the tree. Thus, three combinational trees as follows were input DIVA program:

Tree 1: (((frutex, sinica), rosea), (((arborescens, pekinensis), (boisii, stipitata)), bicolor));

Tree 2: (((frutex, sinica), rosea), ((arborescens, (pekinensis, (boisii, stipitata))), bicolor));

Tree 3: (((frutex, sinica), rosea), ((pekinensis, (arborescens, (boisii, stipitata))), bicolor)).

For these three trees, three different trees added different areas combination on the nodes, could be obtained using DIVA program. Some optimal criteria of minimizing of the dispersal and extinction (Ronquist, 1996, 1997; Nordlander et al., 1996; Xiang et al., 2001; Fritsch et al., 2001; Davis et al., 2002) were used to determine the ancestors at nodes on trees. Consequently, these area combinations at the nodes were treated as the ancestors to determine the vicariance and dispersal events.

3 Results

3.1 ITS and *trn*L-F

In ITS and *trn*L-F combined data matrix, while the gaps were treated as missing positions, there are total 1 692 bp. Of them ITS sequence data included 693 bp, the variable nucleotide positions were 40, informative positions were 21, *trn*L-F sequence data included 999 bp, the variable nucleotide positions were 34, informative positions were 13.

3.2 Phylogenetic analysis

Using the heuristic search option, exhaustive option and band and branch option in PAUP program, just same one parsimonious tree was found (Fig. 2). Thus, strict tree was certainly this parsimonious tree. The tree's length=114, CI=0.9561, RI=0.9206. The high values of CI and RI to Figure 2 illustrated that the phylogenetic tree reconstruction was available. The bootstrap values were labeled on the nodes. It is clear from bootstrap values that there are three distinctive groups (Table 1) with pane and arrowhead in the phylogenetic tree, i.e., groups with the key characters respectively, Sect. *Caragana* is with pinnate leave and deciduous rachis; Sect. *Bracteolatae* is with pinnate leave and persistent rachis; and Sect. *Frutescentes* is with palmate leave and persistent rachis.

3.3 Biogeographical analysis

3.3.1 Component analysis (CA)

Under Assumption 0, three equal parsimonious trees and a strict consensus tree were presented in Figure 3. Under Assumption 1 and 2 945 and 58 equal parsimonious trees were obtained respectively, but the strict consensus trees for both were all shown the paraphyletic relationship to six areas (Fig. 3e). So, these results under Assumption 1 and 2 were unavailable to area relationship.

3.3.2 Brooks parsimony analysis (BPA)

Based on the data matrix and using the Hennig 86 program, the area relationships were illustrated by two equal parsimonious trees and a strict consensus tree were presented in Figure 5.

3.3.3 Dispersal and vicariance analysis (DIVA)

For different ancestral area combination on the nodes of three trees from DIVA results (Fig. 6), the ancestors were determined according to some optimal criteria and minimize the dispersal and extinction. For Tree 1 and Tree 3, the vicariance events at nodes were very similar, especially in the deep node of the trees both were same as “ACF, ABCF, BCDEF, ABCDEF”, and both have four dispersals. The final ancestors at the node and dispersals were illustrated in Figure 7 to Tree 1 and Tree 3, and Figure 8 to Tree 2.

For Tree 2, large vicariance process was beginning from BCF. The first vicariance was between B and CF, and then vicariance between C and F, five dispersal events in this pattern (Fig. 8). For Tree 1 and Tree 3, the vicariance process was beginning from BCDEF, the vicariance events orderly between B and CDEF, between CDE and F (Fig. 7). Because topology of Tree 1 and Tree 3 was different from that of the original phylogenetic tree (Fig. 2), and ancestor of *C. pekinensis*, *C. boisii* and *C. stipitata* was C (broken line, Fig. 7), and C was the ancestor not including *C. arborescens*, therefore, the distribution of *C. arborescens* in C was explained as a dispersal event. Total four dispersal events occur in this process.

Finally, two explanatory processes of vicariance and dispersal (Fig. 7 and Fig. 8) were summed in Figure 9.

4 Discussion

4.1 Phylogenetic relationship

According to the ITS DNA phylogeny (Zhang et al., 2009), three distinctive monophyletic groups (sections) respectively in *Caragana* were recognized, i.e., Sect. *Caragana* with pinnate foliage and deciduous rachis, Sect. *Frutescentes* with palmate foliage and persistent sclerotic stick rachis, and Sect. *Bracteolatae* is with pinnate leave and persistent rachis. The difference

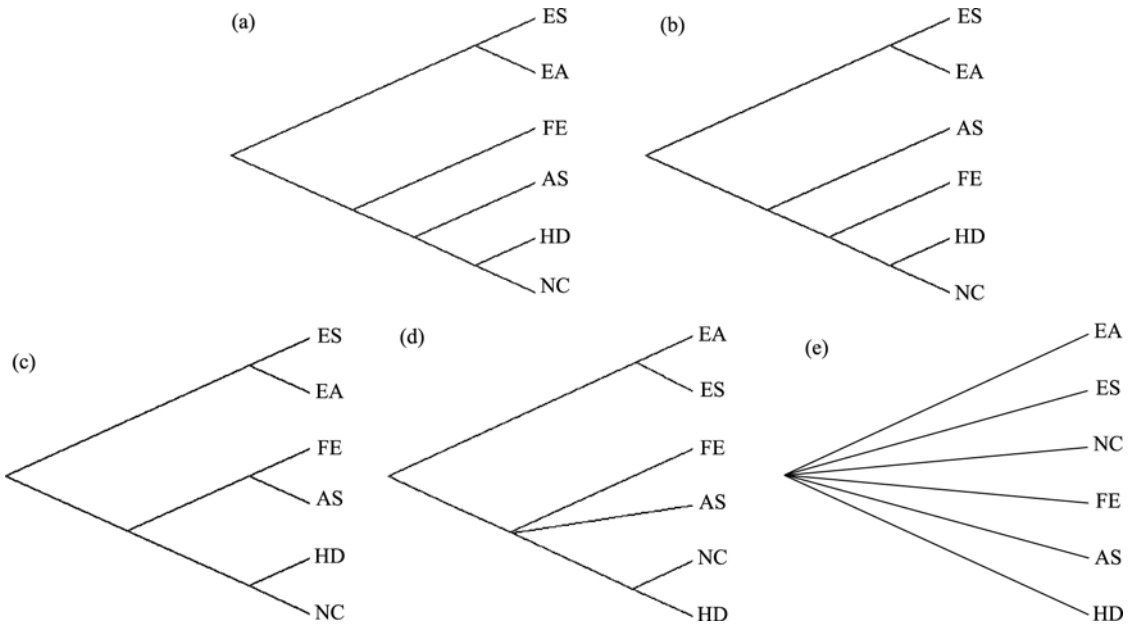

Fig. 3 Area cladograms were obtained under Assumption 0, 1 and 2 by using component analysis (CA). Fig. 3a-d was under Assumption 0, Fig. 3d was the strict consensus tree of Fig. 3a, 3b and 3c. Fig. 3e was respectively strict consensus tree under Assumption 1 and 2.

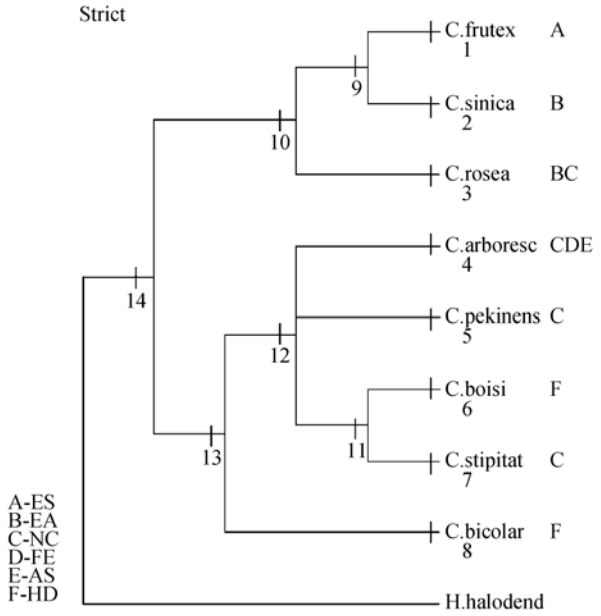

Fig. 4 Based on the phylogenetic tree Fig. 2, the taxa and nodes were labeled 1-14 in order to treat the characters of areas into a matrix for the analytical program need of Hennig 86 to Brooks parsimony analysis (BPA).

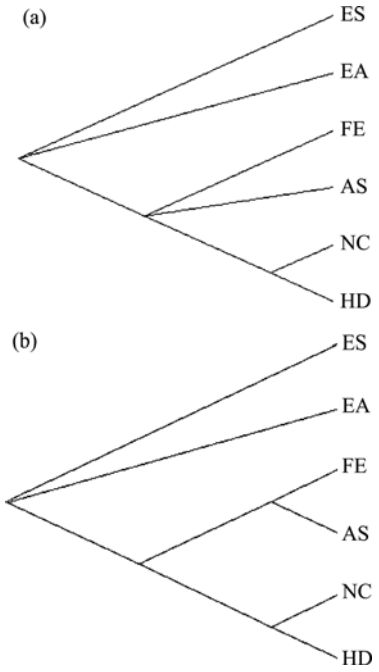

Fig. 5 Area cladograms obtained by using Brooks parsimony analysis (BPA), Fig. 5a and 5b were equal parsimonious trees, and Fig. 5a was also the strict consensus tree for the both.

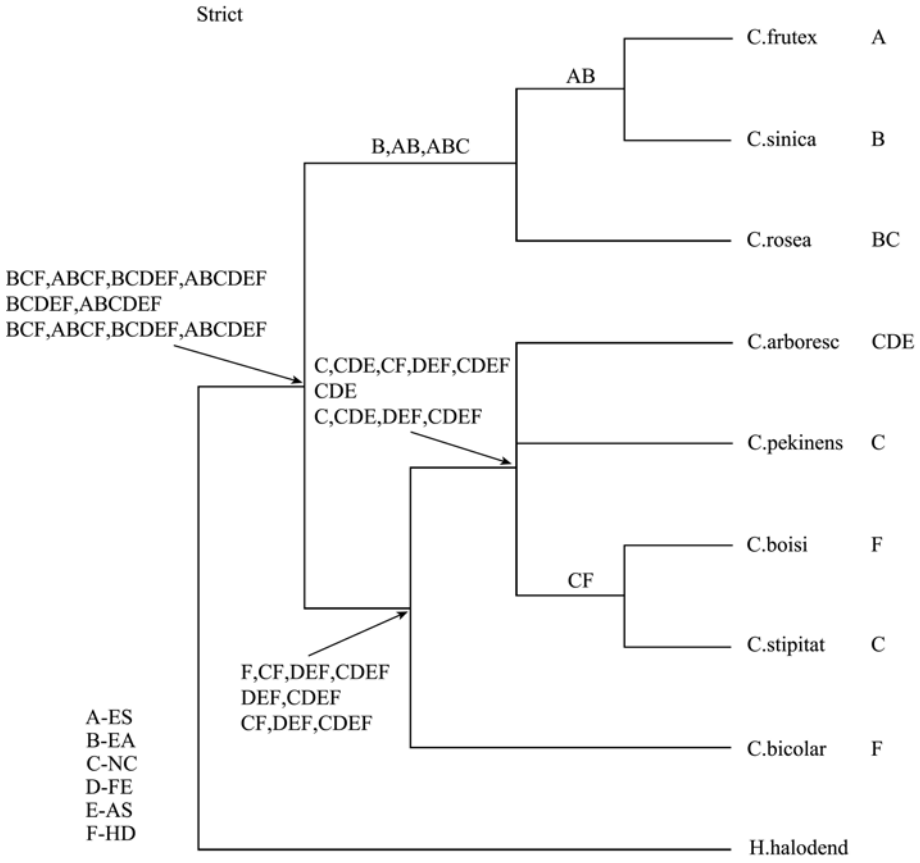

Fig. 6 Dispersal-vicariance analysis (DIVA) result indicated the ancestral area combinations at the nodes on the basis of phylogenetic tree (Fig. 2). Three trees were input to program since a paraphyletic node in Fig. 2, consequently, three ancestral area combinations especially with 3 rows respectively at 3 three nodes using arrowheads were labeled.

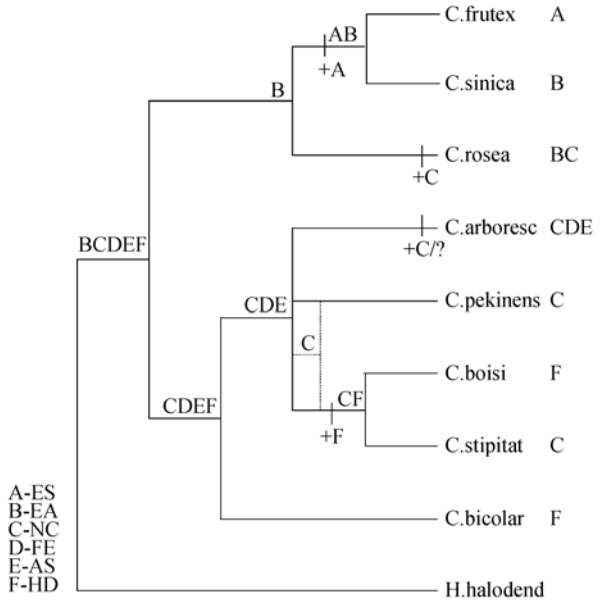

Fig. 7 A biogeographical scenario to explain the vicariance and dispersal, four dispersals occur in this pattern, areas combinations at tree nodes were yielded from Tree 1 (first row combination at 3 nodes using arrowhead) and Tree 3 (third row combination at 3 nodes using arrowhead) in Fig. 6.

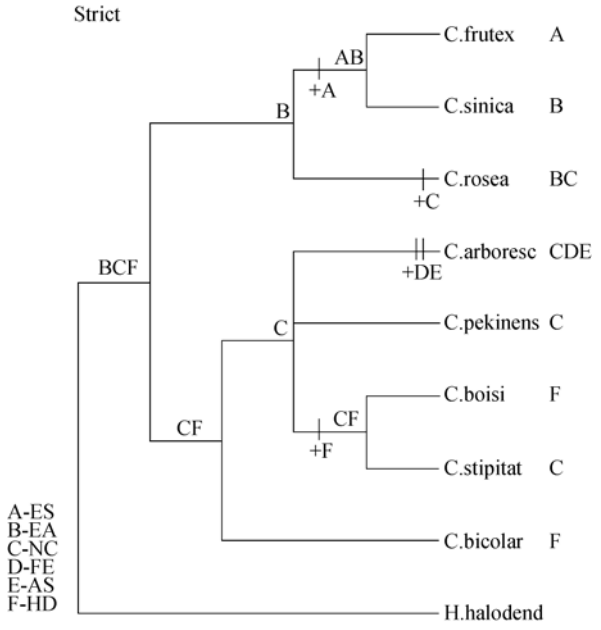

Fig. 8 A biogeographical scenario to explain the vicariance and dispersal, five dispersals occur in this pattern, areas combinations at tree nodes were yielded from Tree 2 (second row combination at 3 nodes using arrowhead) in Fig. 6.

of Sect. *Caragana* and Sect. *Frutescentes* was clearly shown in the phylogenetic tree of this paper (Fig. 2), especially group Sect. *Caragana* with pinnate foliage and deciduous rachis obtained a 100% bootstrap support. The foliage and rachis characters were used

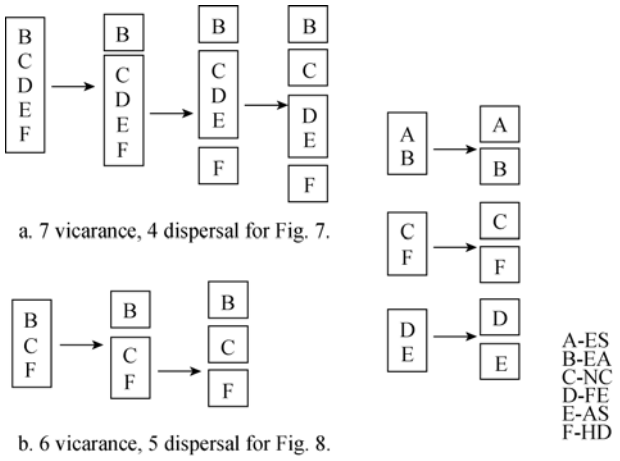

Fig. 9 A summary of DIVA results Fig. 7—8, includes two explanatory processes of vicariance and dispersal. Fig. 9a includes 7 vicariance and 4 dispersal, Fig. 9b includes 6 vicariance and 5 dispersal, right: three vicariance of AB, CF and DE respectively are common to DIVA results of Fig, 7 and Fig. 8.

not only to construct phylogeny especially in Moore's (1968) two-dimension phylogenetic scheme based on the morphological and chromosome evidences, but also as stable key characters to identify infrageneric groups especially at section level in *Caragana* (Komarov, 1908, 1945; Pojarkova, 1945; Moore, 1968; Gorbanova, 1984; Sanchir, 1980, 1999; Liu, 1993; Zhao, 1993; Zhang, 1997b; Zhang et al., 2009). Therefore, the results from both ITS and *trn*L-F sequence data in this paper show again the significance of the characters to phylogeny and classification.

C. bicolor, belonging to Sect. *Bracteolatae* which mainly occurs in Hengduan Mountains, Qinghai-Xizang (Tibet) Plateau and Himalayas, represents another group different from two monophyletic groups mentioned above. In morphology, *C. bicolor* with pinnate foliage and persistent sclerotic stick rachis, possess some characters different from other groups in *Caragana*. So it was suggested an intermediate group between two monophyletic groups mentioned, but its intermediate position was not very clear, in other words, which monophyletic group close to is not ascertained. However, in the present study, *C. bicolor* is fairly illustrated that it has more relationship to Sect. *Caragana*, the group with pinnate foliage and deciduous rachis (Fig. 2) and distributes mainly in North China, Altai-Sayan, and Far East-Northeast China. And the taxon relationships shown in Figure 2 essen-

tially result in the area relationships among six areas. Two groups of Sect. *Caragana* and Sect. *Bracteolatae* in Figure 2. have close relationship, consequently, two areas North China and Hengduan Mountains occurring two groups respectively, have also close area relationship.

4.2 Area relationship

Although six areas are merely selected from 13 areas in the generic distribution, but they have representative of East Asia and Tethys as mentioned above (Zhang 1998). And since these six areas possess some key species, such as *C. arborescens*, *C. boisii*, and *C. bicolor* etc., the area relationship yielded from analytical biogeography can be discussed as follows.

From component analysis (CA) (Fig. 3), the area relationship could be illustrated for (ES, EA, (FE, AS, (NC, HD))). But on the other hand, the area relationship was shown by ((ES, EA), (FE, AS, (NC, HD))) from Brooks parsimony analysis (BPA). Both trees have a same structure (FE, AS, (NC, HD)). The difference was that ES and EA were either paraphyletic using CA or cluster group using BPA to (FE, AS, (NC, HD)). The same structure (FE, AS, (NC, HD)), in fact, expressed an area relationship among four species of Sect. *Caragana*, and North China (NC) had a close relation to Hengduan Mountains (HD) (Fig. 2). The relationship of ES and EA was actually shown by the palmate leave group (Fig. 2) Sect. *Frutescentes*, the close relation of two areas was virtually resulted from the relation of *C. sinica* and *C. frutex*. Therefore, the area relationship in this paper could be clearly determined by that of the species occured. And from area relationships using CA and BPA, the close relationships not only happen between neighbor areas such as between North China (NC) and Hengduan Mountains (HD), but also between far-distance areas, such as East Asia Subtropical (EA) and Europe-West Siberia (ES).

4.3 Vicariance and dispersal

4.3.1 Vicariance versus dispersal plays important role According to the vicariance and dispersal results indicated on the node of trees in Figs. 7—9, vicariance versus dispersal is essential in the differentiation process. The ancestors mainly include BCF, CF, BCDEF, CDEF, CDE, AB, and DE etc., then they are divided into segments. These vicariances generally occur in large regions and isolated areas. For instance in Fig.7 and Fig. 9a, A(ES) and B(EA) formed a large isolated vicariance geographically, i.e. vicariance of East Asia Subtropical (EA) and Europe-West Siberia. C(NC) North China, D(FE) Far East-Northeast China, and E(AS) Altai-Sayan, formed isolated vicariances from their ancestor CDE, which also is isolated geographically. These patterns and events imply vicariance should be better explanation instead of dispersal since dispersal couldn't be possible across so far away isolated areas. At the same time, most species in the genus are endemic, only a few species occur in two or three areas among 13 areas (Zhang, 1998), this also indicates the dispersal should be only a minority, in fact 4 dispersals in Figs. 7 and 5 dispersals in Fig. 8. Therefore, vicariance versus dispersal would be concluded to play important role in *Caragana* distribution pattern.

Hengduan Mountains (F) should been focused on the basis of the flora, ecology and *Caragana* distribution. It has its special flora within China and the North Temperature zone (Wu, 1988). For *Caragana* distribution, Sect. *Bracteolatae* is endemic to Hengduan Mountains, Qinghai-Xizang (Tibet) Plateau and Himalayas (Zhang, 1997a). Morphologically, the taxa in this section are with pinnate foliage 5—9 pair leaflets and persistent sclerotic stick long-thick rachis, which is different from other groups in *Caragana* (Zhang, 1997a). Concerning the flora, vegetation, soil, altitude, precipitation and temperature especially its cold and wet environmental status (Wu, 1979, 1980), Hengduan Mountains has a remarkable difference with its adjacent areas especially North China, as well as Central Asia and other areas. Therefore, it has a possibility of occurring vicariance among Hengduan Mountains and its adjacent areas. In the present paper, vicariance (vicaraince of FC) was shown between it and adjacent North China from Figs. 7—9.

Therefore, there are two vicariance types in *Caragana*. First type is resulted from the isolated areas. An example is areas of *C. arborescens* distribution, Far East—Northeast China, Altai-Sayan and North China. And three related species resulted in isolated distribution areas, East Asia Subtropical with *C. sinica*, East Asia Subtropical and North China with *C. rosea*, Europe—West Siberia with but *C. frutex*. Another vicariance type occurs in adjacent areas. A distinct case is in Figs. 7—9, the vicariance occurs between Hengduan Mountains and North China.

4.3.2 The congruence between species, morphology, DNA sequence and areas

According to the taxon cladograms Fig. 2 and area cladograms Fig. 3 and Fig. 5, there is an obvious congruence between taxon cladogram and area cladogram. In general, the species names in the taxon cladogram are substituted by their occurring areas at the starting stage of biogeographical analysis. Because most species are not widespread except *C. arborescens* and *C. rosea* in Fig. 2, therefore, the area cladograms are virtually ascertained by the taxon cladogram, namely, by the species relationship in phylogenetic tree. The phylogenetic tree in this paper was reconstructed by using the DNA sequence data, it means the gene evidence provides the foundation to phylogenetic tree. Three groups of the phylogenetic tree (Fig. 2) are attributed by the morphological characters, consequently, these congruence could be regarded as the congruence between gene, morphology, species and area (Page, 1993,1994).

4.3.3 Dispersal

Because the most dispersal occurred only between adjacent areas in *Caragana*, for instance, in Figure 8, such as *C. rosea* is dispersal from B (East Asian Subtropical) to C (North China), *C. boisii* is from C (North China) to F (Hengduan Mountains), therefore, the dispersals in this paper should be considered as only short dispersal.

4.4 Phytogeographical evolutionary trend

Komarov (1908, 1945) pointed out that *Caragana* origin place was one location of East Asia on the basis of his hypothesis that *C. sinica* was the primitive species in the genus, this species just occurs in East Asia subtropical region. According to Komarov, from East Asia distribution area, the generic phytogeographical evolutionary trend was postulated westward via Himalayas and Mongolia to Central Asia. A DIVA analytical result of Fig. 8 just provides a sound scenario to explain Komarov's idea. In this result, the root ancestor of *Caragana* was BCF. F (Hengduan Maintains) had only one species *C. bicolor* distribution, and had a vicariance with B (East Asia Subtropical) C (North China). Therefore, BC in fact played the ancestor to most species in the tree. As the areas, both B and C were classified into East Asia region according to the floristic division (Wu, 1979; Wu and Wu, 1999) and vegetation division (Wu, 1980). In addition, F was also classified into East Asia region (Wu and Wu, 1999). Anyway, ancestor either BCF or BC is always included in East Asia. Therefore, Kamarov' idea of East Asia origin place could be explained fairly by DIVA analysis. Of course, Komarov' point is only one of many hypotheses for *Caragana* origin.

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References

Brooks D R. Parsimony analysis in historical biogeography and coevolution: methodology and theoretical update. Systematic Zoology, 1990, 39: 14–30.

Brooks D R, van Veller, MG P, et al. How to do BPA, really. Journal of Biogeography, 2001, 28: 345–358.

Davis C C, Fritsch P W, Li J, et al. Phylogeny and biogeography of *Cercis* (Fabaceae): evidence from nuclear ribosomal ITS and chloroplast *ndf*F sequence data. Systematic Botany, 2002, 27: 289–302.

Donoghue M J, Bell C D, Li J. Phylogenetic patterns in northern hemisphere plant geography. International Journal of Plant Science, 2001, 162(Suppl.6): 41–52.

Doyle J J, Doyle J L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulltin, 1987, 19: 11–15.

Farris J S. Hennig 86 reference, version 1.5. New York. 1988.

Fritsch P W, Morton C M, Chen T, et al. Phylogeny and biogeography of the Styracaceae. International Journal of Plant Sciences, 2001, 162(Suppl. 6): 95–116.

Gorbanova N N. De generis *Caragana* Lam. (Fabaceae) notae systematicae. Novitates Systematicae Plantarum Vascularium, 1984, 21: 92–100.

Humphries C J, Parenti L R. Cladistic biogeography. 2nd ed. London: Oxford University Press. 1999.

Jønsson K A, Fjeldsa J. Determining biogeographyical patterns of dispersal and diversification passerine birds in Australia, Southeast Asia and Africa. Journal of Biogeography, 2006, 33: 1155–1165.

Kang Y, Zhang M L, Chen Z D. A preliminary phylogenetic study of the subgenus *Pogonophace* (Astragalus) in China based on ITS sequence data. Acta Botanica Sinica, 2003, 45: 140–145.

Komarov V L. Generis *Caragana* monographia. Acta Horti Petropolitani, 1908, 29(2): 77–388.

Komarov V L. Opera selecta. Mosquae: Academic Science Press of URSS. 1945. 159–342.

Lavin M, Wojciechowski M F, Richman A, et al. Identifying tertiary

radiations of Fabaceae in the Greater Antilles: alternatives to cladistic vicariance analysis. International Journal of Plant Sciences, 2001, 162 (Suppl.6): 53–76.

Liu Y X. Caragana. In: Flora Reipublicae Sinicae. Beijing: Science Press, 1993, 42(1): 17–67.

Moore R J. Chromosome numbers and phylogeny in *Caragana* (Leguminosae). Canadian Journal of Botany, 1968, 46: 1513–1522.

Nelson G, Platnick N I. Systematics and biogeography; cladistics and vicariance. New York: Columbia University Press. 1981.

Nordalander G, Liu Z, Ronquist F. Phylogeny and historical biogeography of the cynipoid wasp family *Ibaliidae* (Hymenoptera). Systematic Entomology, 1996, 21: 151–166.

Page R D M. Component users manual. Release 1.0. Auckland. 1989.

Page R D M. Genes, organisms, and areas: the problem of multiple lineages. Systematic Biology, 1993, 42: 77–84.

Page R D M. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. Systematic Biology, 1994, 43: 58–77.

Pojarkova A I. Caragana. In: Komarov V L (eds). Flora of USSR. Moscow and Leningrad: Academic Science Press of URSS, 1945, 11: 327–368.

Polhill R M. Galegeae. In: Polhill R M, Raven P H. (ed.) Advances in legume systematics. Part 1. Kew: The Royal Botanical Garden, 1981. 357–363.

Renner S. Plant dispersal across the tropical Atlantic by wind and sea currents. International Journal of Plant Sciences, 2004, 165(Suppl.4): 23–33.

Ronquist F. DIVA, version 1.1. Computer program and manual available by anonymous FTP from Uppsala University. htttp://ftp.uu.se. 1996.

Ronquist F. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Systematic Biology, 1997, 46(1): 195–203.

Sanchir C H. Genus *Caragana* Lam. Systematics, geography, phylogeny and economic significance in study on flora and vegetation of P. R. Mongolia. Vol.1. UlanBotaor: Academic Press. 1979.

Sanchir C H. Outline of *Caragana* Lam. Species. Ulan Bator: Institute of Botany, P. R. Mongolia Academy, 1980, 4: 106–123.

Sanchir C H. System of the genus *Caragana* Lam. (Fabaceae). Acta Scientiarum Naturalium Universitatis Neimongol, 1999, 30(4): 501–512.

Sanderson M J, Liston A. Molecular phylogenetic systematics of Galegeae, with special reference to Astragalus. In: Crisp M D, Doyle J J (ed.). Advances in Legume Systematics. Part 7. Kew: The Royal Botanical Gardens, 1995: 331–350.

Sanderson M J, Wojciechowski M F. Diversification rates in a temperate Legume clade: Are there so many species of *Astragalus* (Fabaceae)? American Journal of Botany, 1996, 83: 1488–1502.

Spalik K, Downie S R. The evolutionary history of *Sium sensu* lato (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. American Journal of Botany, 2006, 93: 747–761.

Swofford D L. PAUP ver. 4.0. Phylogenetic analysis using parsimony. Sunderland: Sinauer Associates. 1998.

Tablerlet P, Gielly L, Pauton G, et al. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology, 1991, 17: 1105–1109.

Thompson J D, Higgins D G, Gibson T J, et al. Improving sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weigh matrix choice. Nuclear Acids Research, 1994, 22: 4673–4680.

Wang F, Li D Z, Yang J B. Molecular phylogeny of Lardizabalaceae based on *trn*L-F sequence and combined chloroplast data. Acta Botanica Sinica, 2002, 44: 971–977.

White T J, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (ed.), PCR protocols: A guide to methods and application. San Diego: Academic Press, 1990: 315–322.

Wen J. Evolution of eastern Asian-eastern North American biogeographyic disjunctions: a few additional issues. International Journal of Plant Sciences, 2001, 162(Suppl.6): 117–122.

Wiley E O.Parsimony analysis and vicariance biogeography. Systematic Zoology, 1988, 37: 271–290.

Wojciechowski M F, Sanderson M J, Hu J M. Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA *trn*L intron data. Systematic Botany, 1999, 24: 409–437.

Wu C Y. China vegetation. Beijing: Science Press, 1980.

Wu Z Y. The regionalization of Chinese flora. Acta Botanica Yunnanica, 1979, 1(1): 1–22

Wu Z Y. Hengduan Mountains flora and her significance. Journal of Japanese Botany, 1988, 63(9): 297–311.

Wu Z Y, Wu S G. A proposal for a new floristic kingdom (realm)—the *E. Asiantic* Kingdom, its delineation and characteristics. In: Zhang A L, Wu S G (eds.). Floristic characteristics and diversity of East Asian plants. Beijing: China Higher Education Press and Berlin: Springer-Verlag, 1999: 3–42.

Xiang Q Y, Soltis D E. Dispersal-vicariance analyses of intercontinental disjuncts: historical biogeographical implications for angiosperms in the Northern Hemisphere. International Journal of Plant Sciences, 2001, 162 (Suppl.6): 29–39.

Zandee M, Roos M C. Component-compatibility in historical biogeography. Cladistics, 1987, 3: 305–332.

Zhang M L. A premilinary analytical biogiography in *Caragana* (Fabaceae). Acta Botanica Yunnanica, 1998, 21(1): 1–11.

Zhang M L. The geographical distribution of the genus *Caragana* in Qinghai-Xizang Plateau and Himalayas. Acta Phytotaxonomica Sinica, 1997a, 35(2): 136–147.

Zhang M L. A reconstructing phylogeny in *Caragana* (Fabaceae). Acta Botanica Yunnanica, 1997b, 19(4): 331–341.

Zhang M L. Ancestral area analysis of the genus *Caragara* (Leguminosae). Acta Botanica Sinica, 2004, 46(3): 253–258.

Zhang M L, Fritsch P W, Cruz B C. Phylogeny of *Caragana* (Fabaceae) based on DNA sequence data from *rbc*L, *trn*S–*trn*G, and ITS. Molecular Phylogenetics and Evolution, 2009, 50: 547–559.

Zhang M L, Ladiges P, Nelson G. Subtree, TASS and an analysis of the genus *Caragana*. Acta Botanica Sinica, 2002, 44(10): 1213–1218.

Zhang M L, Tian X Y, Ning J C. Pollen morphylogy and its taxonomic significance of *Caragana* Fabr. (Fabaceae). Acta Phytotaxonomica Sinica, 1996, 34: 397–409.

Zhao Y Z. Taxonomic study of the genus *Caragana* from China. Acta Scientiarum Naturalium Universitatis Neimongol, 1993, 24(6): 631–653.