Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

Biogeosciences Discussions Open Access EGU

Phylogeny of the Stipa and implications for grassland evolution in China: based on biogeographic evidence

Qing Zhang[1*], Junjun Chen[1], Yong Ding[2]

[1] School of Ecology and Environment, Inner Mongolia University, Hohhot, 010021, China

[2] Grassland Research Institute of Chinese Academy of Agricultural Sciences, Hohhot, 010010, China;

* Corresponding author. Email: qzhang82@163.com

Abstract

The evolution of Chinese grassland is a still an important question biogeography. In this study, the phylogeny of 20 Stipa species (extensively distributed in Chinese grassland) was established to explore the origin and dispersal routes of Chinese grassland. It showed that Stipa species originated at 28 MaBP and they started to differentiate in central Inner Mongolia at 22 MaBP. Then, Stipa species expanded along four routes: (1) they expanded from central Mongolia to the Qilian Mountains, Qinghai, and western Tibet at 16 MaBP. They then gradually expanded from western to eastern Tibet from 11-6 MaBP. (2) At 12 MaBP, they expanded from central Inner Mongolia to the Helan Mountains. (3) At 8 MaBP, they expanded from central Inner Mongolia to the Xinjiang area. (4) At 4 MaBP, they expanded from central to eastern Inner Mongolia. Therefore, we could deduce the formation order of Chinese grasslands: central Inner Mongolia > Qilian Mountains > Qinghai > western Tibet > Helan Mountains > Xinjiang > central Tibet > eastern Tibet > eastern Inner Mongolia. We highlight the importance of the uplift of the Qinghai-Tibet Plateau and paleoclimate changes in triggering the origin and evolution of Stipa species and Chinese grasslands.

Keywords: uplift of the Qinghai-Tibet Plateau; paleoclimate; explosive radiation; phylogeny

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

25

26 **1 Introduction**

27 Different regions on earth harbor distinct biological species even though their
28 environment are the same, indicating that the distribution of organisms not only is
29 driven by contemporary ecological factors but may also be closely associated with
30 historical factors (e.g., geological evolutionary history and population evolutionary
31 history) (Medail and Diadema, 2009; Ordonez and Svenning, 2015; Santos et al.,
32 2017). Based on molecular clocks, the genetic information of existing species could
33 be used to explore the biogeographic processes including the origin, divergence,
34 expansion, and isolation of organisms at the molecular level during all important
35 geological history events. In addition, the time of divergence between closely related
36 species could be basically confirmed (Tedesco et al., 2017; Voskamp et al., 2017;
37 Wang et al., 2017a).

38 Grasslands are an important component of the global ecosystem and provide
39 significant ecosystem functioning and service.. One sixth total land area is covered by
40 grassland (Scurlock and Hall, 1998). The Chinese grasslands, starting from the
41 Northeast China Plain, Inner Mongolian Plateau, Ordos Plateau, and Loess Plateau to
42 the southern margin of the Qinghai-Tibet Plateau, are major part of the Eurasian
43 Steppe, which is the world's largest grassland (Addison and Greiner, 2016; Wu et al.,
44 2015). The Chinese grasslands are of great interests in ecological studies, including
45 grassland biodiversity (Buergi et al., 2015; Yuan et al., 2016), community assembly
46 (Li and Wu, 2016; Niu et al., 2016), stoichiometry (Wang et al., 2017b), and
47 ecosystem function (Jing et al., 2015; Mao et al., 2017). Meanwhile, a few studies
48 have also discussed the origin and dispersal routes of Chinese grassland genus (Favre
49 et al., 2016; Luo et al., 2016). However, the formation and evolutionary processes of
50 Chinese grasslands are still rarely studied.

51 According to paleogeographical climate change, Chinese grasslands might first
52 emerge in the late of the Tertiary Period because of the global cooling and
53 aridification (Wu et al., 2015). In addition, Meang and McKenna (1998) found that
54 perissodactyl-dominant faunas of the Eocene were abruptly replaced by

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

rodent/lagomorph-dominant faunas of the Oligocene in Mongolia Plateau at 33 MaBP, similar to the European *Grande Coupure*. And they think the turnovers were mainly driven by global climatic shifts and prominent biotic reorganization from forest to grassland. Based on the evidence of sporopollen, more studies focused on vegetation shift and climate change in steppe area of China during the mid-late Holocene (Mischke et al., 2016; Shen et al., 2008). These studies are of great importance for revealing the origin and evolution of Chinese grasslands. However, they were mainly based on a certain history period, and did not explain the expansion routes of Chinese grasslands. We need more direct evidence to obtain a comprehensive and continuous history of grassland evolution. Based on currently molecular information, which providing more accurate and direct evidence for the evolutionary process, biogeography can be used to deduce the origin and expansion routes of Chinese grasslands and to explore the effects of important geological historical events (Favre et al., 2016; Ferreira et al., 2017).

Although Chinese grasslands is vast and covers different vegetation types, Stipa species are dominant through the whole Eurasian steppe, including Chinese grasslands (Durka et al., 2013; Hamasha et al., 2012). Because of differences in climate, Stipa species show obvious zonal distribution characteristics in Chinese grasslands. *Stipa baicalensis*, *Stipa grandis*, and *Stipa krylovii* are constructive species in the typical grasslands; *Stipa tianschanica* and *Stipa glareosa* are distributed extensively in the desert steppe; and *Stipa purpurea* has a very extensive distribution in alpine steppes. In addition, *Stipa basiplumosa* and *Stipa subsessiliflora* are also distributed in the high mountains of desert areas (Durka et al., 2013; Wu et al., 2015). Therefore, Stipa species play very important roles in the formation of Chinese grasslands. The evolutionary processes of Chinese grasslands are closely related to the evolution of Stipa species. Studies on the history of origin, divergence and expansion routes of Stipa species are an excellent indicator to reveal the evolution of Chinese grasslands. Therefore, in this study, Stipa species that were distributed extensively in different grassland areas including Inner Mongolia, Qinghai, Tibet, and Xinjiang were collected. The four gene fragments of chloroplasts, *matk*, *rbcl*, *trnh-psba*, and *trnl-f*,

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

were selected to estimate the divergence time tree of Stipa species using BEAST (Bayesian evolutionary analysis by sampling trees). In addition, the origin, divergence and expansion routes of Stipa species were reconstructed based on RASP (Reconstruct Ancestral State in Phylogenies). Finally, the origin and evolutionary processes of Chinese grasslands were deduced and the effects of paleoclimate and geological historical events were further explored.

2 Materials and Methods

2.1 Materials

This study contained 20 species of Stipa species collected from seven different grasslands area (Fig 1, Table 1). *Helictotrichon schellianum*, *Achnatherum splendens*, and *Ptilagrostis pelliotii*, which are closely related to Stipa species, were used as the outgroup (Hamasha et al., 2012). Considering there is certain hybridization between *Stipa* species(Gonzalo et al., 2012), nuclear-derived markers were not employed, and the four gene fragments of chlorophyll, *matk*, *rbcl*, *trnh-psba*, and *trnl-f*, were used for analyses.

2.2 Methods

2.2.1 Total DNA extraction, PCR amplification, and sequencing

This study performed total DNA extraction using the Plant Genome Extraction Kit (Tiangen Biotech,Beijing, China). DNA samples were diluted to 30 ng/μL for future use(Ramirez et al., 2017). The four selected gene fragments were amplified by PCR. The universal primers were acquired from previous studies (Durka et al., 2013; Hamasha et al., 2012; Ren et al., 2011). The PCR reaction volume was 50 μL; specifically, 2 μL of DNA template, 2 μL each of upstream and downstream primers, 25 μL of 2 × PfuUltra Master Mix, and 24 μL ddH_2O were combined (Lu et al., 2013). The *matk* gene amplification was carried out using the following procedure: pre-denaturation at 94 ℃ for 5 min; 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 40 ℃ for 30 s, and extension at 72 ℃ for 1 min; and a final extension at 72 ℃ for 10 min. The same amplification procedure was used for the *trnh-psba* sequence. For the amplification of *trnl-f* and *rbcl*, the annealing temperatures were

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

changed to 57 ℃ and 52 ℃, respectively, and the other conditions remained unchanged. The amplification products were subjected to 1% agarose gel electrophoresis and sent to the Beijing Genomics Institute for sequencing when the samples were ready.

2.2.2 Sequence comparison

The molecular data obtained from sequencing were assembled using BioEdit and compared using ClustalW. The sequence length, conserved sites, and GC content were analyzed using MEGA5.0. These sequence data have been submitted to the GenBank databases under accession number (see appendices).

2.2.3 Divergence time tree construction using BEAST

The combined sequences of the four gene fragments were used for divergence time tree construction Therefore, incongruence length difference (ILD) analysis (heterogeneity test) on the combined gene fragments was required. This analysis was performed using PAUP (Matthews and Rosenberger, 2008). The results showed that P>0.01, indicating that these fragments did not have an obvious conflict and could be used for combined analysis (Rix and Harvey, 2012). In addition, the model selection software jModeltest (Posada, 2008) was used to calculate the screening of the optimal model; the selected model was GTR+G. Because there was no suitable Stipa fossil or gene fragment evolution rate, the average evolution rate of chloroplast gene of herbaceous plants (3.46×10-9s/s/y) was used to calculate the divergence times. First, the model was operated for 100 million generations in BEAST (Drummond and Rambaut, 2007). After the operation was finished, TRACER V1.6 (Rambaut and Drummond, 2013) was used to look up the ESS value. If the ESS value was larger than 200, the result was considered reliable. Finally, the final divergence time tree was checked in FigTree (Rambaut, 2009), which provided the divergence time for Stipa species.

2.2.4 Reconstruction of historical distribution areas of Stipa species based on RASP

This study used the RASP software to deduce the ancestral distribution areas of internal nodes in the tree. The Chinese grasslands were divided into seven sections: (A) eastern Inner Mongolia, (B) central Inner Mongolia, (C) the Helan Mountains, (D)

the Qilian Mountains, (E) Qinghai, (F) Tibet, and (G) Xinjiang. The S-DIVA analysis used all 100 trees and combined trees in the Bayesian collection. A total of 2500 random trees were selected in the analysis. The maximum number of distributions in each distribution area was set as two, and the remaining settings were the default ones (Lu et al., 2013).

3 Results

3.1 Results of sequence feature analysis

The combined analyses of the four gene fragments showed that the length of the combined gene fragments was 4093 bp; 2988 bp were conserved sites, 1102 bp were mutation sites, 227 bp were parsimony-informative sites, and 847 bp were single information sites. The percentage of G+C in the whole sequence was 37.5%, which was much lower than the A+T content.

3.2 Divergence time of Stipa species

The results showed that the divergence time between Stipa species and the outgroup (*Achnatherum splendens*, *Helictotrichon schellianum*, and *Ptilagrostis pelliotii*) was 28.42 MaBP (Oligocene period) based on the joint matrix estimate (Fig. 2). The divergence time of Stipa species was 22.10 MaBP (early Miocene period). There is a explosive rapid radiation of Stipa species around 6.0 MaBP (Fig.2).

3.3 Analysis of reconstruction of ancestral distribution areas of Stipa species using RASP

RASP analyses showed that Stipa species originated and diverged from the outgroup at 28 MaBP (Fig. 3). At node ① (22 MaBP), Stipa species began to differentiate in central Inner Mongolia. The current distribution pattern of Stipa species was formed through 16 expansions and 13 isolated divergences, and the process was more complicated. At node ② (18 MaBP), Stipa species first expanded to near the Qilian Mountains, Qinghai. Subsequently, at node ③ (13-11 MaBP), there was a stronger interaction between the top and bottom of mountains in the Qilian Mountains and other areas in Qinghai. At node ④ (16 MaBP), Stipa species had already expanded from Qinghai to Tibet; however, no large-area expansion

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

occurred immediately. Instead, at node ⑥ (11 MaBP-6 MaBP), Stipa species had an east to west expansion in Tibet. At node ⑤ (12 MaBP), Stipa species expanded along another route from central Inner Mongolia to the Helan Mountains. At node ⑦ (8 MaBP), the expansion route was from central Inner Mongolia to Xinjiang. Finally, at node ⑧ (4 MaBP), the expansion route of the Stipa species was from central to eastern Inner Mongolia.

3.4 Important influences of paleoclimate and geological historical events on the evolutionary history of Stipa species

Fig 4 shows that Stipa species underwent three larger expansion and isolation events. The first larger expansion occurred around 22 MaBP, the second larger expansion occurred approximately 19-16 MaBP, and the third larger expansion occurred around 12-10 MaBP. The peak values of isolation events and expansion events of Stipa species were basically matched. The peak values had certain relationships with the second uplift of the Qinghai-Tibet Plateau and paleoclimate change, including East Asian monsoon formation and polar ice cap development.

4 Discussion

4.1 Origin and differentiation of Stipa species

Mountain building may trigger the origin and radiation of species by providing vacant niches and habitat alternatives within a short distance (Favre et al., 2016; Hoorn et al., 2013; Sun et al., 2012), for example in Andes (Hughes and Atchison, 2015), Himalaya-Hengduan Mountains (Luo et al., 2016) and uplift of the Qinghai-Tibet Plateau (Sun et al., 2012). During the second (23-15 MaBP) and the third (since 8 MaBP) uplift periods of the Qinghai-Tibet Plateau, many species groups, including *Nannoglottis* (Liu et al., 2002), Chinese sisorid catfishes (Guo et al., 2006), *Rheum* (Sun et al., 2012) and *Gentiana* (Favre et al., 2016). Therefore, the uplift of the Qinghai-Tibet Plateau plays important role in the origin and divergence of many species.

In this study, the results showed that Stipa species differentiated from the outgroup and originated at 28 MaBP and began to differentiate at 22 MaBP in central Inner Mongolia (Fig.3b). It is consistent with a previous study showing that Stipe

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

species were at least originated in the Miocene or Pliocene through fossil evidence in North American (Thomasson, 1978). During this period, Himalayan movement had already uplifted the Qinghai-Tibet Plateau to the height above 2000 m that had a critical function in the formation of monsoon circulation (Molnar et al., 1993; Tapponnier et al., 2001). With the continuous uplift and expansion of the plateau, summer sea surface pressure on the Asian continent increased continuously. With the presence of a monsoon climate, the humid climate that used to penetrate from Inner Mongolia to northern Xinjiang no longer existed. This area became particularly arid, which provided possibilities for the origin and first expansion of Stipa species.

Around 6.0 MaBP, there is a dichotomous relationship among these Stipa species, and the internodes are consistently short relative to the average tip nodes (Fig.2). These two results both provide clear indications of explosive rapid radiation in the past (Hughes and Atchison, 2015; Sun et al., 2012). We speculate that this is the results of the third uplift of the Qinghai-Tibet Plateau occurred during the 9-2.61 MaBP period (Molnar et al., 1993; Tapponnier et al., 2001). There may be two major speciation mechanisms that caused the explosive rapid radiation of Stipa species at that time. On one hand, the first speciation mechanism may be allopatric speciation (Boucher et al., 2016). Due to the crumpling effect of the uplift of the Qinghai-Tibet Plateau, the Tian Shan, Qilian, Altyn Tagh, and Kunlun Mountains all had a large-scale elevation of fault blocks, and many areas that were already elevated became medium-height mountains with around 4000 m height (Tapponnier et al., 2001). During this period, geographic isolation was great obvious. As long as there was obvious geographic isolation, species groups were divided into several small species groups; because there was no continuous gene flow, different new species would be generated (Lee and Lin, 2012; Tedesco et al., 2017). On the other hand, for the area without obvious geographic isolation, such as the Inner Mongolian plateau, we considered ecological speciation occurred in the presence of gene flow (Shafer and Wolf, 2013). The uplift of the Qinghai-Tibet Plateau would have caused larger climate fluctuations in Inner Mongolia Plateau (Tapponnier et al., 2001), thus gradually generated different small geographic environments. These different small geographic

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

environments would cause species to occupy different ecological niches, resulting in natural or sexual selection, which would have caused individuals of the ancestral species group to undergo phenotype divergence to generate new species (Greve et al., 2017; Shafer and Wolf, 2013). Therefore, the explosive rapid radiation of Stipa species around 6.0 MaBP was the result of with and without gene flow driven by geographic isolation and climate changes in different region. In addition, analysis of their molecular characteristics showed that the G+C content accounted for 37.5% of the total sequence length, which was much lower than the A+T content. A and T are connected and expanded by two hydrogen bonds; therefore, they are more prone to mutations than G and C (Lee et al., 2016). In addition to external environmental factors, the molecular characteristics of Stipa species also made the occurrence of rapid divergence possible.

4.2 Influence of the Qinghai-Tibet Plateau uplifts and paleoclimate changes on the origin and evolution of the grasslands

During the developmental process of the whole geological history, various geological history events continuously occur such as large tectonic movements, the rise and fall of sea levels, magmatic activities, and volcanic eruptions. Climate changes on earth are closely associated with these geological history events, whereas the development of the biosphere is directly influenced by climate and terrain changes (Guo et al., 2002; Molnar et al., 2010). In China, one of the geological history events that had great influences on the biosphere were several larger uplift events of the Qinghai-Tibet Plateau. After the collision between the Indian and European continents, the Qinghai-Tibet area entered into a whole new developmental stage consisting of mainly orogenic, fault, and magmatic activities (Tapponnier et al., 2001). There were three main stages of uplift. The first stage occurred before 30 MaBP (Turner et al., 1993), the second stage occurred from 23-15 MaBP, and the third stage occurred at 8 MaBP (Molnar et al., 1993). The uplift of the Qinghai-Tibet Plateau caused dramatic climate changes on earth at that time, and the climate became dry and cold; these changes were conducive to the origin and evolution of grasslands (Turner et al., 1993; Wu et al., 2015).

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

Stipa species started to differentiate at 22 MaBP in central Inner Mongolia; therefore, during the same period, this area already had a preliminary grassland landscape. This grassland vegetation structure was also consistent with rodent/lagomorph-dominant mammal faunas of the Oligocene in Mongolia Plateau based on fossil evidence (Meng and McKenna, 1998). Afterward, when the expansion of Stipa species occurred at 16 MaBP, grassland landscapes also emerged successively in the Qilian Mountains, Qinghai, and western Tibet areas. The major event of geological history at this stage was the second uplift of the Qinghai-Tibet Plateau. In addition, factors such as the expansion of rifts in the Asian marginal sea and the partial shrinkage and disappearance of the eastern extension of the Neo-Tethys Ocean in Central Asia all resulted in the formation of the Neogene monsoon climate (Huang et al., 2003; Wang et al., 2003). After it developed at 22 MaBP, the monsoon climate continuously strengthened and became strongest at 16 MaBP. The strengthening of the monsoon climate and aridification of the inland areas were synchronous, thereby causing the further expansion of the arid areas in northwestern China. These events all provided appropriate environmental conditions for the formation and expansion of the grasslands (Guo et al., 2002; Wang et al., 2008). The late Miocene period in geological history occurred approximately 12 MaBP. During this period, the Asian, and even the global, environment also underwent significant changes. The Arctic area had a large amount of ice rafts, the polar ice cap developed further, the climate became drier and colder, an outbreak of C_4 plants occurred, and C_3 plants decreased rapidly (Cerling et al., 1997). Based on these events, central Tibet and the ancient Helan Mountains in Inner Mongolia also exhibited grassland landscapes. During the period of 9-2.61 MaBP, the third uplift of the Qinghai-Tibet Plateau occurred. The uplift of the Qilian Mountains, Tian Shan Mountains, Altyn Tagh Mountains, and Kunlun Mountains made the inland areas more arid (Molnar et al., 1993). During this period, western Tibet, Xinjiang, and eastern Inner Mongolia also successively exhibited grassland landscapes. The uplift of the Qinghai-Tibet Plateau and changes in paleoclimate jointly promoted the origin of grasslands.

5 Conclusion

In summary, Stipa species originated at 28 MaBP and they started to differentiate in central Inner Mongolia at 22 MaBP. Then, Stipa species expanded along four routes. Based on the expansion route of Stipa species, we deduced that the Chinese grasslands formed in the following order: central Inner Mongolia > Qilian Mountains > Qinghai > western Tibet > Helan Mountains > Xinjiang > central Tibet > eastern Tibet > eastern Inner Mongolia. In addition, the origin and evolution of Stipa species and Chinese grasslands were accompanied by the uplift of the Qinghai-Tibet Plateau and paleoclimate change.

6 Author contributions

Q.Z. conceived the ideas. Q.Z. and J.C. complied the experiment and ran further data analysis. Q.Z., C.L. and Y.D. collected study material . Q.Z. and J.C. led the writing.

7 Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8 Acknowledgements:

We are grateful to professor Frank Yonghong Li and Morigen, for proposing some valuable suggestions in experimental design. We also grateful professor Cunzhu Liang for collected some study material. This study was supported by the National Natural Science Foundation of China (31560180) and China Postdoctoral Science Foundation.

References

Addison, J. and Greiner, R.: Applying the social-ecological systems framework to the evaluation and design of payment for ecosystem service schemes in the Eurasian steppe, Biodivers. Conserv., 25, 2421-2440, 2016.

Boucher, F. C., Zimmermann, N. E., and Conti, E.: Allopatric speciation with little niche divergence is common among alpine Primulaceae, J. Biogeogr., 43, 591-602, 2016.

Buergi, M., Li, L., and Kizos, T.: Exploring links between culture and biodiversity: studying land use intensity from the plot to the landscape level, Biodivers. Conserv., 24, 3285-3303, 2015.

Cerling, T. E., Harris, J. M., Macfadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., and Ehleringer,

J. R.: Global vegetation change through the Miocene/Pliocene boundary, Nature (London), 389, 153-158, 1997.

Drummond, A. J. and Rambaut, A.: BEAST: Bayesian evolutionary analysis by sampling trees, BMC Evol. Biol., 7, 2007.

Durka, W., Nossol, C., Welk, E., Ruprecht, E., Wagner, V., Wesche, K., and Hensen, I.: Extreme genetic depauperation and differentiation of both populations and species in Eurasian feather grasses (Stipa), Plant Syst. Evol., 299, 259-269, 2013.

Favre, A., Michalak, I., Chen, C. H., Wang, J. C., Pringle, J. S., Matuszak, S., Sun, H., Yuan, Y. M., Struwe, L., and Muellner-Riehl, A. N.: Out-of-Tibet: the spatio-temporal evolution of Gentiana (Gentianaceae), J. Biogeogr., 43, 1967-1978, 2016.

Ferreira, M., Aleixo, A., Ribas, C. C., and Santos, M. P. D.: Biogeography of the Neotropical genus Malacoptila (Aves: Bucconidae): the influence of the Andean orogeny, Amazonian drainage evolution and palaeoclimate, J. Biogeogr., 44, 748-759, 2017.

Gonzalo, R., Aedo, C., and Garc á, M. Á.: Two New Combinations in Stipa sect. Smirnovia (Poaceae), Ann. Bot. Fenn., 48, 159-162, 2012.

Greve, C., Haase, M., Hutterer, R., Rödder, D., Ihlow, F., and Misof, B.: Snails in the desert: Species diversification of Theba (Gastropoda: Helicidae) along the Atlantic coast of NW Africa, Ecology and Evolution, 2017.

Guo, X. G., He, S. P., and Zhang, Y. G.: Phylogeny and biogeography of Chinese sisorid catfishes re-examined using mitochondrial cytochrome b and 16S rRNA gene sequences (vol 35, pg 344, 2005), Mol. Phylogen. Evol., 38, 291-291, 2006.

Guo, Z. T., Ruddiman, W. F., Hao, Q. Z., Wu, H. B., Qiao, Y. S., Zhu, R. X., Peng, S. Z., Wei, J. J., Yuan, B. Y., and Liu, T. S.: Onset of Asian desertification by 22 Myr ago inferred from loess deposits in China, Nature, 416, 159-163, 2002.

Hamasha, H. R., von Hagen, K. B., and Roeser, M.: Stipa (Poaceae) and allies in the Old World: molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages, Plant Syst. Evol., 298, 351-367, 2012.

Hoorn, C., Mosbrugger, V., Mulch, A., and Antonelli, A.: Biodiversity from Mountain Building, Nature Geoscience, 6, 154-154, 2013.

Huang, R. H., Zhou, L. T., and Chen, W.: The progresses of recent studies on the variabilities of the East Asian monsoon and their causes, Advances in Atmospheric Sciences, 20, 55-69, 2003.

Hughes, C. E. and Atchison, G. W.: The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains, New Phytol., 207, 275-282, 2015.

Jing, X., Sanders, N. J., Shi, Y., Chu, H., Classen, A. T., Zhao, K., Chen, L., Shi, Y., Jiang, Y., and He, J.-S.: The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate, Nature Communications, 6, 2015.

Lee, A.-W., Hsu, C.-C., Liu, Y.-Z., Wei, P.-L., and Chen, J.-K.: Supermolecules of poly(N-isopropylacrylamide) complexating Herring sperm DNA with bio-multiple hydrogen bonding, Colloids and Surfaces B-Biointerfaces, 148, 422-430, 2016.

Lee, Y.-H. and Lin, C.-P.: Pleistocene speciation with and without gene flow in Euphaea damselflies of subtropical and tropical East Asian islands, Mol. Ecol., 21, 3739-3756, 2012.

Li, S. W. and Wu, J. S.: Community assembly and functional leaf traits mediate precipitation use efficiency of alpine grasslands along environmental gradients on the Tibetan Plateau, Peerj, 4, 2016.

Liu, J. Q., Gao, T. G., Chen, Z. D., and Lu, A. M.: Molecular phylogeny and biogeography of the

Qinghai-Tibet Plateau endemic Nannoglottis (Asteraceae), Mol. Phylogen. Evol., 23, 307-325, 2002.
Lu, L., Wang, W., Chen, Z., and Wen, J.: Phylogeny of the non-monophyletic Cayratia Juss. (Vitaceae) and implications for character evolution and biogeography, Mol. Phylogen. Evol., 68, 502-515, 2013.
Luo, D., Yue, J. P., Sun, W. G., Xu, B., Li, Z. M., Comes, H. P., and Sun, H.: Evolutionary history of the subnival flora of the Himalaya-Hengduan Mountains: first insights from comparative phylogeography of four perennial herbs, J. Biogeogr., 43, 31-43, 2016.
Mao, W., Felton, A. J., and Zhang, T.: Linking Changes to Intraspecific Trait Diversity to Community Functional Diversity and Biomass in Response to Snow and Nitrogen Addition Within an Inner Mongolian Grassland, Frontiers in Plant Science, 8, 2017.
Matthews, L. J. and Rosenberger, A. L.: Taxon Combinations, Parsimony Analysis (PAUP*), and the Taxonomy of the Yellow-Tailed Woolly Monkey, Lagothrix flavicauda, Am. J. Phys. Anthropol., 137, 245-255, 2008.
Medail, F. and Diadema, K.: Glacial refugia influence plant diversity patterns in the Mediterranean Basin, J. Biogeogr., 36, 1333-1345, 2009.
Meng, J. and McKenna, M. C.: Faunal turnovers of Palaeogene mammals from the Mongolian plateau, Nature, 394, 364-367, 1998.
Mischke, S., Lai, Z., Long, H., and Tian, F.: Holocene climate and landscape change in the northeastern Tibetan Plateau foreland inferred from the Zhuyeze Lake record, Holocene, 26, 643-654, 2016.
Molnar, P., Boos, W. R., and Battisti, D. S.: Orographic Controls on Climate and Paleoclimate of Asia: Thermal and Mechanical Roles for the Tibetan Plateau. In: Annual Review of Earth and Planetary Sciences, Vol 38, Jeanloz, R. and Freeman, K. H. (Eds.), Annual Review of Earth and Planetary Sciences, 2010.
Molnar, P., England, P., and Martinod, J.: Mantle dynamics, uplift of the Tibetan Plateau, and the Indian Monsoon, Rev. Geophys., 31, 357-396, 1993.
Niu, K., He, J.-S., Zhang, S., and Lechowicz, M. J.: Grazing increases functional richness but not functional divergence in Tibetan alpine meadow plant communities, Biodivers. Conserv., 25, 2441-2452, 2016.
Ordonez, A. and Svenning, J.-C.: Geographic patterns in functional diversity deficits are linked to glacial-interglacial climate stability and accessibility, Global Ecol. Biogeogr., 24, 826-837, 2015.
Posada, D.: jModelTest: Phylogenetic model averaging, Mol. Biol. Evol., 25, 1253-1256, 2008.
Rambaut, A.: FigTree v1.3.1. 2006-2009, 2009.
Rambaut, A. and Drummond, A. J.: Tracer V1.6, 2013.
Ramirez, J. L., Birindelli, J. L. O., and Galetti, P. M., Jr.: A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data, Mol. Phylogen. Evol., 107, 308-323, 2017.
Ren, H., Lu, L.-M., Soejima, A., Luke, Q., Zhang, D.-X., Chen, Z.-D., and Wen, J.: Phylogenetic analysis of the grape family (Vitaceae) based on the noncoding plastid trnC-petN, trnH-psbA, and trnL-F sequences, Taxon, 60, 629-637, 2011.
Rix, M. G. and Harvey, M. S.: Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: Evidence for Miocene speciation within Tertiary refugia, Mol. Phylogen. Evol., 62, 375-396, 2012.
Santos, M. F., Lucas, E., Sano, P. T., Buerki, S., Staggemeier, V. G., and Forest, F.: Biogeographical patterns of Myrcia s.l. (Myrtaceae) and their correlation with geological and climatic history in the Neotropics, Mol. Phylogen. Evol., 108, 34-48, 2017.

Scurlock, J. M. O. and Hall, D. O.: The global carbon sink: a grassland perspective, Glob. Chang. Biol., 4, 229-233, 1998.

Shafer, A. B. A. and Wolf, J. B. W.: Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology, Ecol. Lett., 16, 940-950, 2013.

Shen, C., Liu, K.-B., Morrill, C., Overpeck, J. T., Peng, J., and Tang, L.: Ecotone shift and major droughts during the mid-late holocene in the central Tibetan Plateau, Ecology, 89, 1079-1088, 2008.

Sun, Y., Wang, A., Wan, D., Wang, Q., and Liu, J.: Rapid radiation of Rheum (Polygonaceae) and parallel evolution of morphological traits, Molecular Phylogenetics & Evolution, 63, 150-158, 2012.

Tapponnier, P., Xu, Z. Q., Roger, F., Meyer, B., Arnaud, N., Wittlinger, G., and Yang, J. S.: Geology - Oblique stepwise rise and growth of the Tibet plateau, Science, 294, 1671-1677, 2001.

Tedesco, P. A., Paradis, E., Leveque, C., and Hugueny, B.: Explaining global-scale diversification patterns in actinopterygian fishes, J. Biogeogr., 44, 773-783, 2017.

Thomasson, J. R.: Epidermal patterns of the lemma in some fossil and living grasses and their phylogenetic significance, Science, 199, 975-977, 1978.

Turner, S., Hawkesworth, C., Liu, J., Rogers, N., Kelley, S., and Calsteren, P. V.: Timing of Tibetan uplift constrained by analysis of volcanic rocks, Nature, 364, 50-54, 1993.

Voskamp, A., Baker, D. J., Stephens, P. A., Valdes, P. J., and Willis, S. G.: Global patterns in the divergence between phylogenetic diversity and species richness in terrestrial birds, J. Biogeogr., 44, 709-721, 2017.

Wang, G.-H., Li, H., Zhao, H.-W., and Zhang, W.-K.: Detecting climatically driven phylogenetic and morphological divergence among spruce (Picea) species worldwide, Biogeosciences, 14, 2307-2319, 2017a.

Wang, P. X., Jian, Z. M., Zhao, Q. H., Li, Q. Y., Wang, R. J., Liu, Z. F., Wu, G. X., Shao, L., Wang, J. L., Huang, B. Q., Fang, D. Y., Tian, J., Li, J. R., Li, X. H., Wei, G. J., Sun, X. J., Luo, Y. L., Su, X., Mao, S. Z., and Chen, M. H.: Evolution of the South China Sea and monsoon history revealed in deep-sea records, Chin. Sci. Bull., 48, 2549-2561, 2003.

Wang, X., Ma, X., and Yan, Y.: Effects of soil C:N:P stoichiometry on biomass allocation in the alpine and arid steppe systems, Ecology and Evolution, 7, 1354-1362, 2017b.

Wang, Y., Cheng, H., Edwards, R. L., Kong, X., Shao, X., Chen, S., Wu, J., Jiang, X., Wang, X., and An, Z.: Millennial- and orbital-scale changes in the East Asian monsoon over the past 224,000 years, Nature, 451, 1090-1093, 2008.

Wu, J., Zhang, Q., Li, A., and Liang, C.: Historical landscape dynamics of Inner Mongolia: patterns, drivers, and impacts, Landscape Ecol., 30, 1579-1598, 2015.

Yuan, Z. Y., Jiao, F., Li, Y. H., and Kallenbach, R. L.: Anthropogenic disturbances are key to maintaining the biodiversity of grasslands, Sci. Rep., 6, 2016.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

456

457 ## Tables

458 **Table 1** Relevant information about samples used in the experiment. The latin name indicates
459 Stipa species latin name. The origin indicates the location of Stipa species. The altitude indicates
460 Stipa species altitude. The latitude and longitude indicates Stipa species latitude and longitude.
461 The area type indicates the partition type of Stipa species in RASP software.

Species name	Latin name	Origin	Altitude (m)	Latitude and longitude	Area type
Stipa baicalensis	*Stipa baicalensis*	Hulunbuir, Inner Mongolia	1650	49°20’57”N 120°07’09”E	A
Stipa krylovii	*Stipa krylovii*	Xilingol League, Inner Mongolia	930	47°51’58”N 115°46’48”E	B
Stipa capillata	*Stipa capillata*	Jungar Banner, Inner Mongolia	895	39°26’27.7”N 111°09’43.2”E	B
Stipa grandis	*Stipa grandis*	Xilingol League, Inner Mongolia	1686	44°30’25.9”N 117°21’47.1”E	B
Stipa breviflora	*Stipa breviflora*	Darhan Muminggan United Banner, Inner Mongolia	1376	41°50’18.1”N 110°13’45.3”E	B
Stipa klemenzii	*Stipa klemenzii*	Saihan Tala	1123	42°48’49.9”N 112°36’18.4”E	B
Stipa glareosa	*Stipa glareosa*	Erenhot, Inner Mongolia	942	43°36’42.2”N 111°59’29.9”E	B`
Stipa tianschanica	*Stipa tianschanica*	Helan Mountains, Inner Mongolia	1715	38°42’49.5”N 105°58’43.7”E	C

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

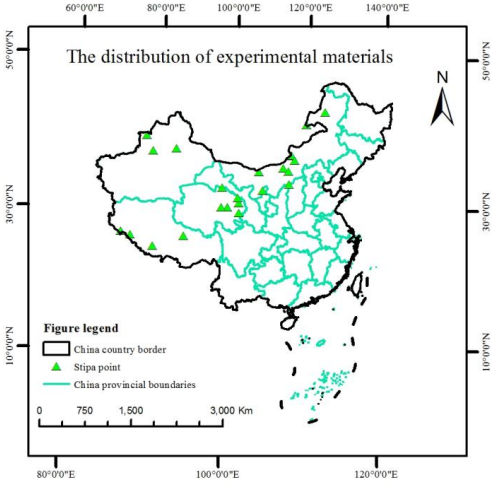
Stipa aliena	*Stipa aliena*	Qilian Mountains, Qinghai	3201	37°36’38.9”N 101°19’31.3”E	D
Stipa penicillata	*Stipa penicillata*	Haibei, Qinghai	3201	37°36’43.7”N 101°19’09.0”E	D
Stipa regeliana	*Stipa regeliana*	Haibei, Qinghai	3220	37°37’04.6”N 101°19’32.3”E	D
Stipa przewalskyi	*Stipa przewalskyi*	Haixi, Qinghai	2936	37°13’09.5”N 101°32’11.8”E	D
Stipa purpurea	*Stipa purpurea*	Haibei, Qinghai	3456	37°03’39.0”N 101°41’55.5”E	E
Stipa orientalis	*Stipa orientalis*	Nagqu, Tibet	4483	31°26’26.6”N 92°01’07.2”E	F
Stipa roborowskyi	*Stipa roborowskyi*	Tibet	4805	30°29’02.3”N 81°10’19.0”E	F
Stipa subsessiliflora	*Stipa subsessiliflora*	Tibet	3444	32°57’24.28”N 95°15’46.05”E	F
Stipa capillacea	*Stipa capillacea*	West Ngamring County, Tibet	4487	29°19’27.27”N 86°58’28.81”E	F
Stipa capillacea	*Stipa basiplumosa*	Tibet	4655	30°20’09”N 82°54’31.6”E	F
Stipa caucasica	*Stipa caucasica*	Sai Hubei, Xinjiang	2170	44°54’03.8”N 81°43’20.9”E	G
Stipa sareptana	*Stipa sareptana*	Xinjiang	1750	49°24’55.1”N 95°26’36.5”E	G
Achnatherum splendens	*Achnatherum splendens*	Xilamuren, Inner Mongolia	1607	41°20’48.5”N 111°10’17.0”E	B
Ptilagrostis pelliotii	*Ptilagrostis pelliotii*	Urad Middle Banner, Inner Mongolia	1518	41°53’36.8”N 107°43’32.2”E	B
Helictotrichon	*Helictotrichon*	Bayanbulak,	2470	42°54’0”N	G

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

schellianum	*schellianum*	Xinjiang	83°43’0”E

462

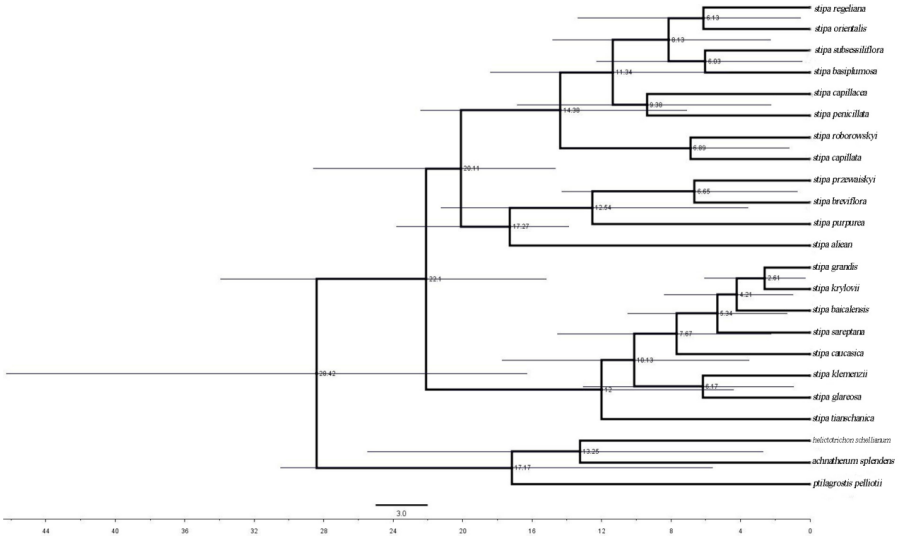
463 ## Figure Legends



464

465 **Figure 1** Distribution of sample material collection points. The green triangle means the
466 distribution of Stipa species. The green line represents the China provincial boundaries. The black
467 frame means China country borders.

468



469

470 **Figure 2** Divergence time of Stipa species based on BEAST (Units: Ma, million years). The right

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

side of the picture is the Latin name of the Stipa species. The number of branches is the divergence time of Stipa species.3.0 means the branch length of divergence time tree. The line segment represents time scale on the bottom of picture.

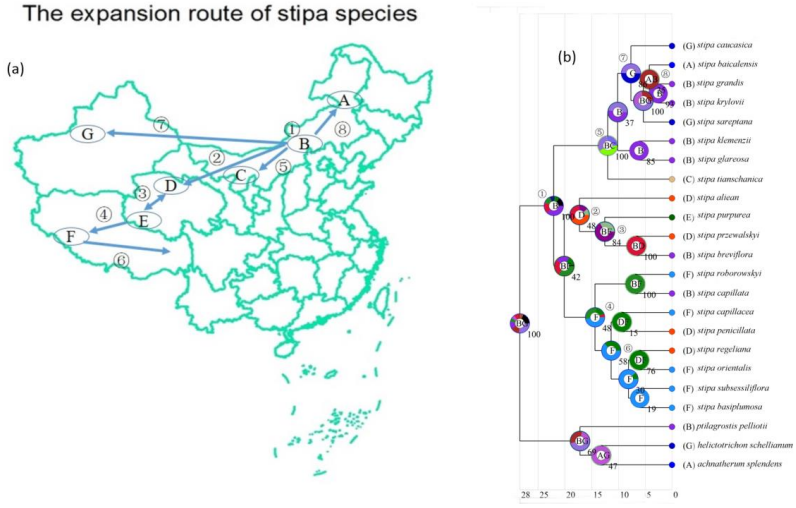


Figure 3 Analysis of ancestral geographic distribution of Stipa species using RASP. In figure 3(a), The Chinese grasslands were divided into seven sections: (A) eastern Inner Mongolia, (B) central Inner Mongolia, (C) the Helan Mountains, (D) the Qilian Mountains, (E) Qinghai, (F) Tibet, and (G) Xinjiang. The one-way arrows indicate the expansion route calculated using RASP, the two-way arrow indicates the phenomenon of mutual expansion between two areas. In figure 3(b), The right side of the picture is the Latin name of the Stipa specie. The letter on each node of the tree represents the largest possible distribution area of Stipa species in the corresponding time period. The line segment represents time scale on the bottom of picture. ①-⑧ in Figs 3a and 3b are landmark nodes of the origin, divergence, and expansion of Stipa species.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-140
Manuscript under review for journal Biogeosciences
Discussion started: 26 April 2018

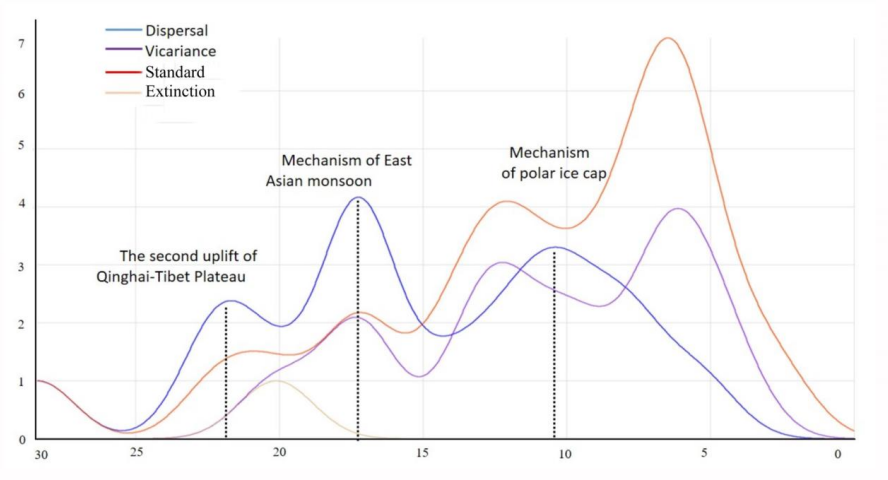


Figure 4 The time-geological time curve. The population dynamics of Stipa species was described by time abscissas and range ordinates. There are four curves in the graph, and the blue curve represents the expansion trend of Stipa species, the purple curve represents the vicariance trend of Stipa species, the pink curve represents extinction of Stipa species, the red curve is the standard curve.