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Editorial

Evolutionary history and underlying adaptation of alpine plants on the Qinghai–Tibet Plateau

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Abstract As the highest and largest plateau in the world, the Qinghai–Tibet Plateau, with its numerous endemic species, is one of the important alpine biodiversity hotspots. Only recently have the evolutionary histories and underlying adaptations of these alpine plants become clear, through research mostly based on testable experiments and analyses. In this issue, we collected a total of 13 papers related to such aims. In addition, we selected a few published papers to highlight the major findings in the recent past. We also outlined the outlook and direction of future research.

1 A brief introduction to the present issue

The Qinghai–Tibet Plateau (QTP) is the highest and largest plateau in the world with an average elevation of more than 4000 m. It started to uplift after the collision between India and Eurasia approximately 50 million years ago (Ma). However, all extensive uplifts occurred only after the early Miocene, between 30–23 Ma, 15–7 Ma, and 3.6–1.6 Ma. The southern QTP may have reached the current elevation earlier than the northern part, but it is still difficult to pinpoint the exact timescale when each part of the QTP reached the current elevation. The number of plant species occurring there varies from 9000 to 12 000, depending on the plateau ranges and altitudes defined by different authors. However, only around 4000 species are found in the high altitude of 4000 m in the interior plateau. More than 20% of the total number of species found in the QTP are endemic although such an estimation also shifts slightly according to different authors and selected ranges. Undoubtedly, the QTP flora comprises one of the important alpine biodiversity hotspots in the world. However, where were these plants from? What adaptations did they make and how have they developed? In addition, due to the topological effects of the QTP, climatic oscillations during the Quaternary should have been enforced there and all plants occurring there consequently should have been driven into extinction, migration, and evolution. How did the alpine species in the QTP respond to the Quaternary climatic oscillations?

Although early researchers mainly centered on describing new species and compiling floristic lists, numerous experiments were designed to address the above questions in the recent past. In this issue, we collected 13 papers related to these topics. First, four studies were designed to examine the origins of the QTP species and floristic compositions of the particular ecological zones. Lu et al. (2014) suggested that *Ostryopsis intermedia* (Betulaceae), endemic to the SW QTP, might have originated through diploid hybrid speciation based on the authors' population genetic analyses of this species and two congeners. This is probably the third homoploid hybrid species reported from the QTP. Chen et al. (2014a) reported the chromosome numbers and karyotypes of 15 species from *Cyananthus* (Campanulaceae) and they found that most species are diploids, whereas only two species were found to be polyploids. This is consistent with previous investigations of other genera (e.g., Liu et al., 1999, 2001; Liu, 2004; Nie et al., 2005). All these studies suggested that most species in the QTP diverged at the diploid level. Therefore, polyploidization contributed little to the total species diversification in the QTP, although polyploid species were found in a limited number of genera. Xu et al. (2014) examined species compositions of the alpine subnival belt in the QTP, which suggested that most species occurring there

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were derived from recent diversifications. Li et al. (2014b) examined phylogenetic structure along the altitudinal gradients and found phylogenetic overdispersion at the low altitudes, but phylogenetic clustering at the high altitude. They suggested that the phylogenetic community was shaped by the environmental filter, interspecies interaction, rapid speciation, and inter-plant distance.

Second, phylogeographic studies of five alpine species were reported in order to examine their responses to the Quaternary climatic oscillations and subsequent evolutions. Wang et al. (2014) found that *Pomatosace filicula* (Primulaceae) probably survived in multiple refugia during the Last Glacial Maximum (LGM) and the earlier Largest Glaciation might have resulted in the deep intraspecific divergences. All these findings suggested that allopatric speciation at the diploid level predominated there. Kou et al. (2014) also found that the differentiated groups of *Hippophaë neurocarpa* (Elaeagnaceae) survived the LGM in multiple locations. But local adaptation to the heterogeneous climates subsequently led to the production of the current clear intraspecific morphotypes or subspecies. An aquatic herb *Ranunculus bungei* survived not only the LGM but probably also previous glacial periods on the QTP (Chen et al., 2014b). Yue & Sun (2014) similarly found multiple refugia for *Spenceria ramalana* (Rosaceae) and they further suggested montane isolation may have promoted the intraspecific divergences of this species. Huang et al. (2014) found such deep divergences have similarly occurred within *Allium wallichii* (Amaryllidaceae), but at the stage when the QTP was extensively uplifted during the Late Pliocene rather than within the Quaternary.

Finally, reproductive adaptations of alpine plants were studied in the following four papers. Guo et al. (2014) examined sexual interference in two *Chamerion* (Onagraceae) species. These two species have contrasted modes of movement herkogamy. The QTP–Himalayan endemic *C. conspersum* has a higher level of interference of stigmas in pollen removal than the widespread *C. angustifolium*. Zhang et al. (2014) found strong inbreeding depression in the self-pollinated *Comastoma pulmonarium* (Gentianaceae) and suggested that selfing is a “better than nothing” choice for alpine plants in harsh environments when there are not enough pollinators. Peng et al. (2014) further examined plant sexual systems in the subnival and alpine regions of the eastern QTP. They found that most species are hermaphroditic, encouraging outcrossing, but they are also self-compatible, which might ensure reproductive success when pollinators are absent. This special sexual system should have optimized the trade-off between genetic variation, gene flow, and reproductive insurances to the greatest degree. Finally, Sun et al. (2014a) reviewed recent research progress in ecological adaptation and reproductive insurance of alpine plants in the harsh habitats of the QTP.

2 Tentative summaries of recent advances

Several papers have reviewed recent advances in the studies of evolutionary history and underlying adaptation of alpine plants on the QTP. For example, Wen et al. (2014) summarized the evolutionary histories of plant diversification, and Liu et al. (2012) reviewed the phylogeographic studies of plants in this region. Here, we further highlight the major findings through selecting a few case studies.

2.1 Summary one: Phylogenetic origin of alpine species in the QTP

Most QTP endemic genera originated *in situ* or in adjacent regions (e.g., Liu et al., 2000; Wang et al., 2007; Zhang et al., 2011b; Zhou et al., 2013), although a few diverged from their sister genera or clades in other regions, far away from the QTP (e.g., Liu et al., 2002; Tu et al., 2010). Similarly, widespread genera occurring in both the QTP and other regions may have diverged from their sister clades in the QTP (e.g., Wang et al., 2004; Zhang et al., 2010; Jia et al., 2012; Li et al., 2014a), adjacent regions (Wang et al., 2007; Zhang et al., 2012), or far away from the QTP (Milne et al., 2010). In most cases, it is difficult to determine the accurate origin location of some species-rich genera due to frequent long-distance dispersals, although they undoubtedly originated in Eurasia (Mao et al., 2010). Most QTP endemic species or clades in species-rich genera were found to originate from radiative diversifications: multiple paralleling descendants from a common ancestor rather than a dichotomous divergence in the phylogenetic tree, based on the molecular evidence (Liu et al., 2006; Wang et al., 2009b; Mao et al., 2010; Sun et al., 2012). These radiations were dated to be largely consistent with the several extensive QTP uplifts from the Miocene to the Pliocene. During the radiative diversification, parallel evolutions with developments of similar or the same traits were commonly found in the examined genera (e.g., Liu et al., 2006; Wang et al., 2009b; Sun et al., 2012). Geological isolations should have also played an important role in such a radiative diversification. In fact, for those genera with fewer species, allopatric speciation through geological isolation contributed greatly to current species diversity (Xu et al., 2010).

The random drift due to fast isolation may have promoted the fixture of the unique characters, for instance, woodiness within the herbal lineages (Tian et al., 2011), and unusual inflorescence (Liu et al., 2000). In addition, interspecific introgression may be more common than previously assumed due to the frequent contacts of the recently diverged species with incomplete reproductive isolations. This hybridization should have further triggered hybrid speciation through both allopolyploid and homoploid speciation. However, allopolyploid speciation is not as common as previously assumed (Liu et al., 2001; Liu, 2004; Nie et al., 2005), despite the fact that polyploid species may have obvious advantages in surviving harsh habitats (Wu et al., 2010). Homoploid speciation is rarely reported in plants, but three diploid hybrid species were confirmed in the QTP (Wang et al., 2001; Song et al., 2003; Ma et al., 2006; Sun et al., 2014b). The relatively common occurrence of homoploid hybrid speciation in the QTP was ascribed to a special combination of frequent interspecific hybridization and newly created habitats by both geological and climatic changes (Sun et al., 2014b). It should be noted that some genera or lineages diversified initially in the QTP and then migrated out of the QTP (Zhang et al., 2009; Jia et al., 2012; Sun et al., 2012; Nie et al., 2013; Li et al., 2014a).

2.2 Summary two: Evolutionary responses to Quaternary climatic oscillations

As found in North America and Europe, a certain haplotype in some species is widespread in the QTP platform and was also found in populations as one of several haplotypes occurring at the plateau edge (Zhang et al., 2005; Meng et al., 2007; Chen et al., 2008; Yang et al., 2008a). Therefore, it is reasonable to hypothesize that these species had retreated to the plateau edge during the glacial ages, and then recolonized the platform during the interglacial ages (e.g., after the Largest Glaciation) and/or at the end of LGM. It remains particularly difficult to determine these species' timescales of recolonization or expansion. In contrast, in some perennial or shrub species, different haplotype groups with distinct divergences were found in different parts of the QTP, even in the high-altitude platform (Wang et al., 2009a; Jia et al., 2011, 2012). Because these divergences pre-dated the LGM, sometimes consistent with the Largest Glaciation (1.2–0.6 Ma), these species undoubtedly survived the LGM in multiple refugia, even at the high-altitude platform. Results based on more nuclear datasets and niche modeled distributions suggested that some cold-preferring conifers might have expanded (rather than shrunk) their distributional ranges during the LGM (Li et al., 2013; Liu et al., 2013b; Sun et al., 2014b). In addition, these studies highlight the importance of the Largest Glaciation, rather than the LGM, in shaping the distributional ranges, genetic diversity, and intraspecific divergences of the current species. This glaciation might also have triggered interspecific introgressions, even the diploid hybrid speciation due to the distributional shifts and second contacts of the closely related species (Sun et al., 2014b). The diverged lineages due to the isolation caused by this Largest Glaciation might have contacted again and led to the intraspecific introgressions (Wang et al., 2009a). It should be noted that the QTP uplifted extensively within the Pliocene and therefore it is difficult to distinguish the geological consequences of the QTP uplift from those by climatic oscillations of the Quaternary (Li et al., 2011a, 2011b). However, interspecific introgressions caused by these Quaternary climatic oscillations may be more frequent in the QTP than previously assumed (e.g., Du et al., 2011; Zou et al., 2012).

2.3 Summary three: Reproductive adaptation to harsh habitats

Most specialized traits or behaviors of alpine plants were found to confer reproductive assurance in harsh habitats. For example, the unusual development of the antipodal cells in the embryo sac may be beneficial to embryo development in alpine habitats (Liu & Ho, 1996), while floral closures in response to both decreasing temperature and pollination similarly increase female and male fitness as well as the seedset and embryo development (He et al., 2005). In fact, this adaptive fitness was also found and tested for in the bracts of glasshouse plants (Wang et al., 2010; Song et al., 2013), dense hairs of downy species (Yang et al., 2008b), and drooping flowers of nodding plants (Chen et al., 2013). The bright flower colors of alpine plants may also benefit them to attract pollinators. Bumblebees were found to pollinate most groups because of their strong ability to fly in the alpine habitats (e.g., Duan et al., 2005; Hong & Li, 2005; Huang & Fenster, 2007; Tang et al., 2007; Zhu et al., 2010; Yu et al., 2012; Yang et al., 2013); however, other insects were also found to play an important role in pollinating alpine plants, especially in the wet eastern QTP (Fang & Huang, 2013). In most pairs of examined species, shifts of pollinators were not found for interspecific divergence and speciation (Huang & Fenster, 2007; but see Liu & Huang, 2013). Closely related sympatric species may have remained partly isolated through being pollinated by different parts of the same pollinator or by different flowering times (Hou et al., 2008; Huang &

Shi, 2013). Wind pollination provides an alternative solution for retaining outcrossing and assuring reproductive success under unfavorable conditions when pollinators are scarce (Duan et al., 2009). In fact, wind pollinates the dominant species of the major ecosystems (e.g., alpine meadow, steppe grassland, conifer forests) in the central and eastern QTP. Self-pollination is another important solution to reduced pollinator diversity and activities due to the low temperatures in high altitudes. In both annuals and perennials, autonomous selfing was found to have provided reproductive assurance under pollinator scarcity (e.g., Zhang & Li, 2008; Duan et al., 2010), even under strong inbreeding depression (Zhang et al., 2011a, 2014). In the subnival region of the SE QTP, more than 85% of the total number of species are hermaphroditic, likely favoring selfing when pollinators are unavailable (Peng et al., 2014).

3 Outlook and direction in the future

If we are to conserve and use plant resources in the QTP effectively, we need to build a solid knowledge of how these evolutionary units were delineated, how they formed, how are used (adapted) to the current alpine habitats, and how they will response to climate changes in the future. Here, we predict how the next generation of biological skills and evolutionary theory will enforce our knowledge in all these aspects. The critical unsolved questions raised and listed here can be used to guide research in the future.

3.1 "Species" delimitation and diversification

"What is a species?" remains debated (Sites & Marshall, 2004). However, "a species" should comprise a separate lineage evolving independently from closely related ones. Although a species may not be monophyletically related to allied ones, it should have developed genetic and morphological gaps from the neighbor species at the population level, although the gaps to define a species need to be balanced within a genus or a family. A genetic gap makes the delimited or defined "species" testable and objective, rather than descriptive or subjective as done before (Harrington & Near, 2012). Therefore, a scientific delimitation of most named species in the QTP needs to be carried out based on both genetic and morphological evidence. Most current species were described based on type specimens without statistical analyses of morphological variations at the population level. Some species might have been established based on single or several mutated individuals within a population, which might be wiped out by the latter selections at the population level. In addition, because of species diversification within short timescales and incomplete reproductive isolations, some species might have been described based on "hybrid" or "introgressed individuals" at the hybrid zones of two closely related species, such as *Rhododendron agastum* (Zha et al., 2010). Due to the decreasing price to obtain genetic data, especially through sequencing DNA barcodes, it is highly desirable to establish genetic gaps to sample multiple individuals to delimitate the species occurring in the QTP. For the recently diverged species, genetic gaps may be too small to be distinguished by the DNA barcodes or a single nuclear locus. However, the differences accumulated from the multiple loci or DNA can together delimitate closely related species with minor morphological differentiation if they do represent different lineages (Niemiller et al., 2012). With the well-delineated "species", we can establish the well-solved genus phylogeny and estimate origin timescale of the QTP endemic species. Finally, meta-analyses of the origin timescales of most endemic species from most genera will illuminate the species diversification history of the QTP flora on a large scale.

3.2 Genetic basis for speciation and adaptation

Although radiative, allopatric, and hybrid speciation patterns were suggested for some groups in the QTP, speciation patterns inferred from these studies had rarely been statistically tested. In fact, it is difficult to distinguish allopatric and parapatric patterns. The dichotomous and radiative divergences are also difficult to discriminate in some scenarios. Using population sequence data, especially from genomic data generated by the Next Generation Sequencer (NGS), and recently developed statistical approaches (e.g., Approximate Bayesian Computation; Li et al., 2013), these alternative hypotheses can be easily distinguished (e.g., Sun et al., 2014b). In fact, such genomic datasets at the population level also provide a solid basis for looking for speciation and adaptation genes. The speciation genes should diverge faster than others and therefore develop genomic divergence islands when a pair of species is compared (Savolainen et al., 2013). The differentiated functions of such diverged alleles (or orthologous genes) and/or new genes, especially their roles in reproductive isolation and reducing interspecific gene flow, should be highlighted and tested by diverse biochemical and molecular approaches. These analyses also pave a way to examine whether these potential speciation genes or the linked genes contributed the observed morphological differentiations between closely related species. For example, if such a pair of sister species or populations are distributed at high and low altitudes, the recovered genes might partly account for the high-altitude adaptations of alpine plants (Li et al., 2011b). For a species distributed along an altitudinal gradient, alleles at the adaptation locus should also change their frequencies accordingly (Savolainen et al., 2013). We predict that the mutations, especially at the regulation regions that result in different alleles, may have contributed more to adaptation and speciation of alpine plants than the mutations at the protein coding regions. It is interesting to know how and when the alleles leading to reproductive isolation developed, for instance, within the ancestral species or after the split of the ancestral species, into the current two species by geographical isolation and/or natural selection. However, we also need to know the relative roles of natural selection versus genetic drift in the different speciation histories. Finally, the molecular mechanisms and parallel evolution of special traits of alpine plants from different lineages, for example, bracts of glasshouse plants (Liu et al., 2013a) and bright-colored flowers, should be further explored to understand their diverse adaptions to their habitats. Such adaptive developments of special traits also promoted species diversification within these genera.

3.3 Biotic interaction

Pollinator–plant interactions need further exploration (Fang & Huang, 2013), especially regarding why fewer insect species pollinate so many plant species in the QTP, how plant species avoid heterospecific pollen disturbance, and how closely related species remain reproductively isolated when the same pollinator is shared. In addition to pollinator–plant interactions, it is also interesting to know how insect or microbial communities change with plant communities, along with changed habitats, and how insects, plants, and microorganisms depend on each another to survive. In past research, the interaction between large animals and plants has been largely neglected. For example, how do plant species respond to extensive grazing by domestic animals (e.g., yaks, sheep, and goats)? Do different plant species have different responses and do these responses shift with altitudinal or other environmental changes? Recently, it was hypothesized that the largest alpine pasture (or meadow) ecosystem in the QTP, composed mainly of the sedge *Kobresia pygmaea*, was controlled "top-down" by grazing yaks or sheep (Miehe et al., 2014). When the grazing animals were excluded, grass species replaced *Kobresia* and the accompanying species. This dynamic change needs to be further monitored and confirmed by independent experiments. Plant–plant interactions comprise interspecific and intraspecific actions. Competition (negative) and facilitation (positive) are two contrasting aspects of such interactions. Intraspecific individual–individual interactions may vary greatly with habits and life history of each species. For most herbal species occurring in harsh habitats, it needs to be explored whether individual–individual actions may be more positive than negative when density is not too high, which facilitates the mutual growth (Chu et al., 2009). Further detailed comparisons about variations or the general pattern of such effects between different species are badly needed. Similarly, whether interspecific facilitation is more important than competition in the alpine habitats also needs further testing. For cushion communities distributed in the scree belt, the facilitation for "parasite" species is obvious; however, it remains unclear what cushion plants benefit from the "parasite" species. In alpine pasture, the dominant *Kobresia* species seem to exclude the growth of other species. Within a confined community, how positive and negative interactions between species reach a trade-off needs further exploration and testing. In addition, each species differs in both genetic and trait variations, which undoubtedly affects their efficiency and metabolism within a community. The community stability and biomass production rely not only on interindividual and interspecific interactions, but also on phylogenetic distances of the composed species (Cadotte et al., 2008; Flynn et al., 2011). Evolutionary processes that shaped the community phylogeny should be further incorporated into such studies of biotic interactions.

3.4 Extinction, survival, and migration

Although many cold-adapted species were found to survive in the central QTP, it remains unknown where the cryptic refugia were located. However, the identification of such small refugia requires more nuclear datasets and computational tests. Therefore, in the future, sequence population genetic data, especially from genomic data by NGS, are needed. These data may not only identify cryptic refugia, but also illuminate historical extinction in local regions and migration routes at the large scale in response to climate changes (Tzedakis et al., 2013). Another aspect of such studies should extend to species with reverse responses (Birks & Willis, 2008). Some cold-preferring species may have survived in high-altitude regions during the interglacial or postglacial periods, but expanded in the QTP and adjacent highlands during glacial ages. These species now should be restricted to the central QTP, but have disjunct populations in the high mountains out of the QTP, therefore suggestive of interglacial or postglacial refugia. It should be noted that the turbulent climatic shifts of the Quaternary not only wiped out diversity, but also created new diversity. The repeated splitting of populations by these climatic oscillations has probably induced intraspecific divergence and cryptic speciation (Wang et al., 2009a; Jia et al., 2011), as well as hybridization, introgression, and hybrid speciation due to long-distance migrations. Two important trends for such studies have appeared and both of them should be enforced together in the future. First, three lines of evidence, phylogeographic surveys based on molecular evidence, fossil records (Birks, 2003), and distributional shifts inferred from species distribution models, should be integrated and reconciled to trace spatiotemporal migration, extinction, and survival of the alpine plants occurring in the QTP. Second, plant ancient DNA preserved in permafrost sediments or within macrofossils at different timescales, recovered through NGS, should be widely applied to infer species extinction and expansion at the local ecosystem (Willerslev et al., 2014). This is especially useful for examining vegetation shifts caused by climatic changes and other factors. All hypotheses of ecosystem and vegetation shifts on the QTP within the Quaternary triggered by both climate changes and humans can be confirmed by this approach. Studying plant extinction, migration, and survival in history can undoubtedly help us predict responses of alpine plants to contemporary climate change and help to conserve them more effectively in the future.

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