

唐古特虎耳草谱系地理学研究

更吉卓玛^{1,2}, 李彦^{1,2}, 贾留坤^{1,2}, 夏铭泽^{1,2}, 高庆波^{1,3*}

(1 中国科学院西北高原生物研究所 中国科学院高山植物适应与进化重点实验室, 西宁 810001; 2 中国科学院大学, 北京 100039; 3 中国科学院西北高原生物研究所 青海省作物分子育种重点实验室, 西宁 810001)

摘要: 利用叶绿体基因(*trnL-trnF* 和 *rpl16*)对青藏高原地区的 18 个唐古特虎耳草(*Saxifraga tangutica* Engl.) 居群(209 个个体)进行谱系地理学研究,以揭示唐古特虎耳草的现有遗传结构及其历史演化过程。结果表明:(1) 从 209 个个体中共检测到 74 个单倍型,且只有单倍型 H5 在居群中广泛分布,71.62% 的单倍型为居群特有单倍型。(2) 分子变异分析(AMOVA)显示,91.85% 的遗传变异来源于居群内,居群间遗传分化不明显($F_{ST} = 0.081$); 遗传分化系数 N_{ST} (0.109) 大于 G_{ST} (0.097, $P > 0.05$) 但不显著,表明唐古特虎耳草在其整个分布范围内没有明显的谱系地理结构。(3) 中性检验表明, Tajima's D (-2.045 07, $P < 0.05$) 和 Fu & Li' D^* 值 (-3.629 27, $P < 0.05$) 均为显著的负值,结合单峰的错配分布曲线,表明该物种经历过近期扩张。研究推测,唐古特虎耳草在第四纪冰期时可能存在多个微型避难所,由于第四纪冰期气候的反复波动,使得原来连续的居群片段化,避难所内的居群单独进化,从而形成了大量特有单倍型;唐古特虎耳草居群对第四纪冰期气候波动的反应可能更多的表现为垂直海拔高度的迁移,而非大规模的水平迁移。

关键词: 唐古特虎耳草; 谱系地理学; 叶绿体 DNA; 遗传多样性; 青藏高原

中图分类号: Q948.2; Q346⁺.4 **文献标志码:** A

Phylogeography of *Saxifraga tangutica* Engl. (Saxifragaceae)

GENGJI Zhuoma^{1,2}, LI Yan^{1,2}, JIA Liukun^{1,2}, XIA Mingze^{1,2}, GAO Qingbo^{1,3*}

(1 Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China; 2 University of Chinese Academy of Sciences, Beijing 100039, China; 3 Key Laboratory of Crop Molecular Breeding of Qinghai Province, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China)

Abstract: Two chloroplast DNA intergenic spacers, *trnL-trnF* and *rpl16*, were employed to study phylogeography of *Saxifraga tangutica*, which aimed to explore genetic structure and phylogeographical history of this species. The result showed that: (1) based on range-wide sampling (18 populations and 209 individuals), we detected 74 haplotypes. Only one haplotype (H5) was widespread, while 71.62% were private haplotypes, i. e., observed in single populations. (2) Analysis of molecular variance showed that within-population variation was described as 91.85% of the total variation, while among-population variation was not distinct ($F_{ST} = 0.081$). An estimation of non-significantly larger N_{ST} (0.109) than G_{ST} (0.097, $P > 0.05$) value across all populations suggested an absence of phylogeographical structure across the whole distribution range. (3) Neutrality tests of Tajima's D (-2.045 07, $P < 0.05$) and Fu & Li' D^* (-3.629 27, $P < 0.05$) showed significantly negative values, in combination with unimodal of mis-

收稿日期: 2017-10-12; 修改稿收到日期: 2017-12-19

基金项目: 中国科学院“西部之光”人才培养引进计划; 中国科学院青年创新促进会项目(2016378)

作者简介: 更吉卓玛(1993-), 女, 在读硕士研究生, 主要从事青藏高原植物适应与进化研究。E-mail: 1018965100@qq.com

* 通信作者: 高庆波, 副研究员, 硕士生导师, 主要从事虎耳草属植物分类学及系统发育学研究。E-mail: qbgao@nwipb.cas.cn

match distribution analysis, suggested a recent demographic expansion of *S. tangutica*. Based on results given above, we speculated that there might be multi microrefugia for *S. tangutica* on the Qinghai-Tibetan Plateau platform during Quaternary glaciations. Climatic oscillations during Quaternary glaciations fragmented the distribution range of *S. tangutica* into isolated populations, subsequent allopatric divergence resulted in the formation of numerous private haplotypes across its distribution range. Populations of *S. tangutica* possibly experienced *in situ* altitudinal migrations to respond glacial and interglacial intervals, instead of extensively horizontal migration.

Key words: *Saxifraga tangutica*; phylogeography; chloroplast DNA; genetic diversity; the Qinghai-Tibetan Plateau

由于复杂的地理拓扑结构、微环境以及更广泛的生态位,高山地区往往具有较高的生物多样性,如高加索山、安第斯山、阿尔卑斯山等^[1-3]。青藏高原及其周边的高山地区包含了 3 个全球生物多样性热点地区,即东部的横断山区、南部的喜马拉雅山系及西部的阿尔泰和天山山脉^[4]。一般认为,与造山运动相关的地理隔离和异域分化是导致青藏高原地区植物多样性较高的原因之一^[5-6]。快速辐射分化在青藏高原地区的很多植物类群中被检测到,如马先蒿属(*Pedicularis* L.)^[7]、红景天属(*Rhodiola* L.)^[8]、风毛菊属(*Saussurea* DC.)^[9]、虎耳草属(*Saxifraga* L.)^[10-11]、橐吾属-垂头菊属-蟹甲草属复合群(*Ligularia* Cass.-*Cremanthodium* Benth.-*Parasenecio* W. W. Sm. & J. Small)^[12]等。然而,与较高分类阶元的分化模式不同,青藏高原地区植物种内居群间的分化可能更多的受到第四纪冰期气候波动的影响。更新世晚期以来,青藏高原经历了至少 4 次规模较大的冰期,其中聂聂雄拉冰期(第二冰期,约 720~500 ka)强度最大,冰川扩张最广,此后冰期强度逐渐减弱^[13-14]。而全球末次盛冰期时青藏高原冰川的扩张程度非常有限,这与欧洲和北美大陆末次冰期形成统一的冰盖不同^[15]。第四纪冰期与间冰期交替所引起的气候波动对于现存生物类群的地理分布格局和遗传结构产生了极大的影响^[16-17]。由于复杂的地理地貌特征、未被统一的大冰盖覆盖以及东亚季风气候和印度季风气候的影响,青藏高原地区的植物可能具有更复杂的第四纪冰期进化历史^[18-19]。一方面,某些植物,如祁连圆柏(*Juniperus przewalskii*)^[20]、青海云杉(*Picea crassifolia*)^[21]等,在第四纪冰期时由高原台面退缩到海拔较低的东南部边缘避难所内,间冰期或冰期后再从边缘避难所回迁到高原台面,从而形成现有的地理分布格局。另一方面,由于第四纪冰期时青藏高原未被统一的大冰盖覆盖,仅有不同程度的山地冰川发育,高原台面可能存在适宜的局部微环境

而成为某些耐寒植物的避难所。对银露梅(*Potentilla glabra*)^[22]、露蕊乌头(*Aconitum gymnanthum*)^[23]和西川红景天(*Rhodiola alsia*)^[24]等的研究揭示,在第四纪冰期时高原台面的居群并没有退缩到高原边缘避难所,而是在高原台面上的一个或几个较大的避难所中保留下来,间冰期或冰期后再由避难所向附近扩散。此外,对西藏圆柏复合群(*Juniperus tibetica* complex)^[25]、西藏沙棘(*Hippophae tibetana*)^[26]、菊叶红景天(*Rhodiola chrysanthemifolia*)^[27]等的研究发现,这些物种第四纪冰期时在高原台面存在多个微型避难所,这些微型避难所在物种的分布范围内呈均匀分布。然而,具有不同分布范围、生境和生活史特征的植物可能具有不同的第四纪冰期进化历史。对青藏高原地区更多的植物进行谱系地理学研究,不但可为高山植物演化历史与第四纪冰期的关系提供依据,同时对青藏高原生物多样性保护具有重要启示。

虎耳草属是虎耳草科(Saxifragaceae)最大的属,约有 450~500 种,主要分布在欧洲和亚洲的高山地区,某些物种环北极分布,另有一些物种扩散到南美洲的落基山脉、安第斯山脉和火地岛^[10-11]。中国产虎耳草属植物约 220 种,主要分布在青藏高原-喜马拉雅地区^[28]。虎耳草属植物被广泛地用于谱系地理学和系统发育学研究,来揭示北极和高山地区植物的分化式样^[10-11, 29-32]。然而,青藏高原作为虎耳草属物种多样性中心之一,该地区虎耳草属植物种内分化与第四纪冰期的关系尚不明确。唐古特虎耳草(*S. tangutica* Engl.)为多年生草本植物,生于海拔 2 900~4 600 m 的高山草甸、灌丛、石隙,分布于青海、甘肃、四川、西藏等地,是青藏高原地区高山草甸的重要组成成分,对维持高寒草甸生态系统的稳定具有重要意义。本研究选取叶绿体 DNA(cpDNA)非编码区 *trnL-trnF* 和 *rpl16* 片段对青藏高原地区的唐古特虎耳草进行谱系地理学研究,目的在于揭示该物种居群内与居群间的遗传分布式

样,讨论唐古特虎耳草对于第四纪气候波动的响应,探讨其现有地理分布格局的历史成因。

1 材料和方法

1.1 实验材料

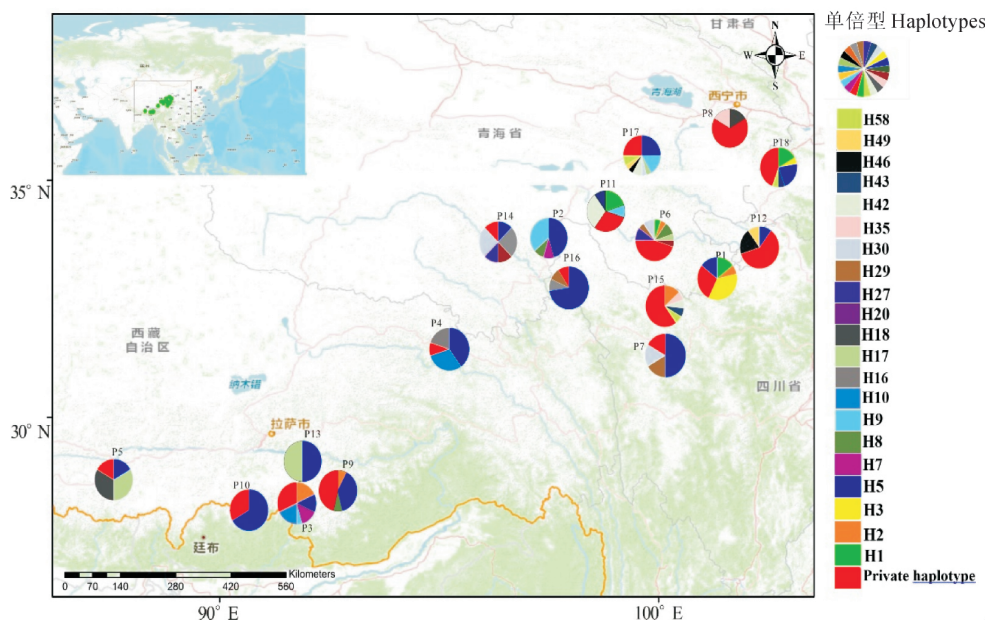
研究材料于 2007 年至 2014 年采自青海、西藏、

四川、甘肃,共采集唐古特虎耳草 18 个居群 209 个个体(表 1,图 1)。每个居群随机采集 2~24 个个体,个体之间相隔至少 10 m 以上。采集生长良好植株的新鲜嫩叶后立即用硅胶干燥,带回实验室存放于-20 °C 冰箱备用。凭证标本由 R. J. Gornall 教授和高庆波副研究员共同鉴定,馆藏于中国科学

表 1 唐古特虎耳草 18 个居群的采集信息

Table 1 Sampling information of 18 populations of *S. tangutica*

| 编号 Code | 凭证标本 Voucher specimen | 采样地 Locality | 纬度 Latitude (N)/° | 经度 Longitude (E)/° | 海拔 Altitude/m | 采样数 Number |
|------------|--------------------------|------------------------|----------------------|-----------------------|------------------|---------------|
| P1 | Chen2014074 | 四川阿坝 Aba, Sichuan | 33.195 3 | 101.47 | 3 680 | 14 |
| P2 | Chen2007020 | 青海称多 Chengduo, Qinghai | 34.020 8 | 97.515 | 4 600 | 11 |
| P3 | Chen2014466 | 西藏错那 Cuona, Xizang | 28.426 6 | 91.880 3 | 5 280 | 22 |
| P4 | Chen2007064 | 西藏丁青 Dingqing, Xizang | 31.691 1 | 94.923 1 | 4 880 | 10 |
| P5 | Chen2007077 | 西藏定日 Dingri, Xizang | 28.932 8 | 87.44 | 5 160 | 6 |
| P6 | Chen2008010 | 青海甘德 Gande, Qinghai | 34.016 9 | 99.999 7 | 4 230 | 20 |
| P7 | Chen2014210 | 四川甘孜 Ganzi, Sichuan | 31.829 1 | 100.276 4 | 4 550 | 6 |
| P8 | Gao2015002 | 青海贵德 Guide, Qinghai | 36.359 8 | 101.447 7 | 3 795 | 6 |
| P9 | Chen2014382 | 西藏隆子 Longzi, Xizang | 28.632 9 | 92.219 2 | 5 120 | 13 |
| P10 | Chen2014482 | 西藏洛扎 Luozha, Xizang | 28.4109 | 90.5754 | 5 110 | 3 |
| P11 | Chens11096 | 青海玛沁 Maqin, Qinghai | 34.5603 | 99.4931 | 4 520 | 10 |
| P12 | Chen2014056 | 甘肃玛曲 Maqu, Gansu | 33.744 8 | 101.875 2 | 3 790 | 10 |
| P13 | Chen2014361 | 西藏乃东 Naidong, Xizang | 28.829 4 | 92.055 | 5 004 | 2 |
| P14 | Chen2014687 | 青海曲麻莱 Qumalai, Qinghai | 33.967 5 | 96.577 4 | 4 570 | 8 |
| P15 | Chen2014162 | 四川色达 Seda, Sichuan | 32.509 7 | 100.389 4 | 4 360 | 15 |
| P16 | Chen2014243 | 四川石渠 Shiqu, Sichuan | 33.081 | 97.962 2 | 4 170 | 11 |
| P17 | Chen2007013 | 青海兴海 Xinghai, Qinghai | 35.514 4 | 99.511 1 | 4 430 | 24 |
| P18 | Chen2014002 | 青海循化 Xunhua, Qinghai | 35.563 9 | 102.713 2 | 1 870 | 18 |



饼状图表示每个居群中单倍型的频率

图 1 唐古特虎耳草 18 个居群的采样图以及叶绿体单倍型分布图

Pie charts show the proportions of haplotypes within each population

Fig. 1 Map of the 18 sampled populations of *S. tangutica* and the distribution of cpDNA haplotypes in the species

院西北高原植物研究所青藏高原生物标本馆(HNWP)。

1.2 DNA 提取与 PCR 扩增

本研究采用改良的 CTAB 法从硅胶干燥的叶片中提取总 DNA^[33]。采用通用引物对所有个体的 cpDNA *trnL-trnF* 和 *rpl16* 片段进行 PCR 扩增^[34-35]。PCR 扩增反应体系为 50 μ L: 包含 5 μ L 的 10 \times PCR Buffer(含 1.5 mmol/L MgCl₂), 2 μ L 的 10 mmol/L dNTPs, 正反引物各 1.0 μ L(5 pmol/L), *Taq* DNA 聚合酶(TaKaRa, 大连)0.4 μ L, 1 μ L 的 DNA 模板, 用双蒸水补足到 50 μ L。PCR 扩增反应程序: 94 $^{\circ}$ C 预变性 10 min; 94 $^{\circ}$ C 变性 1 min, 58 $^{\circ}$ C 退火 50 s, 72 $^{\circ}$ C 延伸 1 min, 32 循环; 72 $^{\circ}$ C 延伸 10 min。PCR 产物用 1% 的琼脂糖凝胶电泳进行检测, 然后送测序公司进行双向测序。

1.3 数据分析

利用 Chromas 2.6.2 软件(<http://www.technelysium.com.au>)对测序峰图进行肉眼核对。利用 MEGA 7.0.26 软件对序列进行对位排列并统计序列的碱基组成^[36], 发现 *trnL-trnF* 序列上存在一段约 20 bp 的 poly A 结构, 为了确保碱基变异的可靠性, poly A 及两侧约 40 bp 的碱基被删除。同样, 对 *rpl16* 序列中出现的大于 11 bp 的 poly A 和 poly T 结构也进行了类似的删除处理。利用 DnaSP 5.10 软件统计 cpDNA 联合序列的变异位点并确定单倍型^[37]。总遗传多样性(H_T)、居群内平均遗传多样性(H_S)、居群间遗传分化系数 G_{ST} 和 N_{ST} 值均利用 PERMUT 软件进行计算^[38]。其中 G_{ST} 仅考虑单倍型的频率, 而 N_{ST} 兼顾了单倍型频率和单倍型之间的相似性。使用 U-统计方法对 G_{ST} 和 N_{ST} 进行比较(1 000 次重复的置换检验)以检测单倍型变异的地理分布模式。 N_{ST} 大于 G_{ST} 且显著, 表明居群在分布范围内具有显著的谱系地理结构。由于 PERMUT 软件要求每个居群至少要有 3 个个体, 所以, 居群 P13(2 个个体)在进行 PERMUT 分析时未包含在内。

运用 Arlequin 3.5.2 计算每个居群的单倍型多样性(H_e)和核苷酸多样性(π)^[39]。利用 Arlequin 软件包中的分子变异分析(AMOVA)检测居群内和居群间的遗传变异水平, 并对单倍型分布的 F_{ST} 进行评价(1 000 次置换检验)^[40]。Tajima's D 、Fu & Li's D^* 两种无限突变位点模型的中性检验及歧点分布分析都在 DnaSP 5.10 程序中完成^[41-42]。

以最大简约法为原则, 利用 NETWORK 4.6.

1.3 软件构建单倍型中央连接网状图^[43-44]。在运行该软件时, 对于多碱基的插入、缺失或突变位点, 被认为是通过一步突变形成。在单倍型 Network 图中, 原始的单倍型多存在于网络图的内部, 而近代起源的单倍型发生在网络图的边缘位置^[45-46]。

2 结果与分析

2.1 唐古特虎耳草的单倍型分布

对 18 个居群 209 个个体的 *trnL-trnF* 和 *rpl16* 片段进行测序, MEGA 软件对位排列后的矩阵长度为 1 626 bp, 通过 DnaSP 软件鉴定出 74 个单倍型, 有 25 个简约信息位点(表 2)。在检测出的 74 个单倍型中, 有 53 个单倍型是居群特有单倍型, 单倍型 H5 出现的频率最高, 有 46 个个体拥有此单倍型。表 3 显示在 18 个居群中, 16 个居群拥有特有单倍型。居群的遗传多样性(H_e)范围为 0.490 9~1.000 0, 平均为 0.853 6; 居群的核苷酸多样性(π)范围为 0.007 506~0.046 481, 平均为 0.022 145。

2.2 居群遗传结构特征

通过 PERMUT 程序计算得出唐古特虎耳草居群内平均遗传多样性 H_S 值(0.843)、总遗传多样性 H_T 值(0.933)、居群间遗传分化 G_{ST} (0.097)和 N_{ST} 值(0.109)。使用 U-统计方法对唐古特虎耳草单倍型变异的地理结构进行检验后发现 N_{ST} 大于 G_{ST} , 但不显著($P > 0.05$), 且居群间的遗传分化水平很低, 表明唐古特虎耳草在现有分布区域内没有明显的谱系地理结构。分子变异分析(AMOVA)的结果表明, 居群间的遗传变异仅为 8.15% ($F_{ST} = 0.081 45$; $P < 0.01$), 而居群内的遗传变异为 91.85%(表 4), 进一步揭示了唐古特虎耳草遗传变异主要存在于居群内。

2.3 基于 cpDNA 联合序列的居群历史动态分析

对唐古特虎耳草 cpDNA 联合序列所有个体进行中性的检验, 结果表明, Tajima's D (-2.045 07, $P < 0.05$)和 Fu & Li's D^* (-3.629 27, $P < 0.05$)均为显著的负值, 推测其分布范围或者个体数近期经历过扩张。歧点分布分析结果显示, 在唐古特虎耳草整个分布范围内观测到的歧点分布呈单峰曲线(图 2), 其 SSD 值(0.007 2)和 Raggednes index 值(0.007 6)均不显著($P > 0.05$), 说明该物种在整个分布范围内经历过近期扩张现象, 这与中性检验结果一致。

2.4 单倍型之间的系统发育关系

最大简约法(maximum parsimony, MP)、最大

表 2 唐古特虎耳草 74 个单倍型 *trnL-trnF* 和 *rpl16* 片段简约信息位点

Table 2 Parsimony informative sites of 74 haplotypes of *S. tangutica* based on *trnL-trnF* and *rpl16* fragments

| 单倍型 Hap | 简约信息位点 Parsimony informative site | | | | | | | | | | | | | | | | | | | | | | | | |
|------------|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|--------------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | <i>trnL-trnF</i> | | | | | | | | <i>rpl16</i> | | | | | | | | | | | | | | | | |
| | 230 | 355 | 414 | 456 | 611 | 792 | 813 | 928 | 966 | 988 | 1000 | 1090 | 1124 | 1192 | 1334 | 1352 | 1359 | 1360 | 1414 | 1451 | 1457 | 1465 | 1479 | 1551 | 1562 |
| H1 | G | T | G | T | A | C | G | T | G | T | G | G | T | C | C | G | G | A | T | T | C | C | G | T | C |
| H2 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H3 | - | C | T | - | - | - | C | C | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H4 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | A | A | - | A | - | T |
| H5 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - |
| H7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H8 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H9 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H11 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | T |
| H14 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H16 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H17 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - |
| H18 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H19 | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H20 | - | - | - | - | - | - | - | - | - | - | - | T | - | A | - | - | - | - | - | - | - | - | - | - | T |
| H21 | - | - | - | - | C | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | G | T | |
| H22 | - | C | - | - | - | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | |
| H23 | - | C | - | - | - | - | A | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | |
| H24 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | T | |
| H25 | - | C | T | C | - | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H26 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H27 | - | - | - | - | C | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | G | T | |
| H28 | - | C | T | C | - | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | |
| H29 | - | C | - | - | - | - | A | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | |
| H30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | |
| H31 | - | C | T | C | - | - | - | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H32 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | T | |
| H33 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H34 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H35 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | |
| H36 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H37 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | T | |
| H38 | - | C | T | C | - | - | - | C | - | C | - | - | - | - | A | - | - | - | - | - | - | - | - | - | |
| H39 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | T | - | T | |
| H40 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | |
| H41 | A | - | - | - | - | T | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | T | |
| H42 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | G | - | - | - | - | - | T | |
| H43 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | A | - | - | - | |
| H44 | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | |
| H45 | - | C | T | C | - | - | - | C | - | C | - | - | - | - | - | - | - | - | - | - | - | T | - | - | |
| H46 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H47 | - | C | T | C | - | - | - | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H48 | - | - | - | - | C | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | G | T | |
| H49 | - | - | - | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | A | A | - | A | T | |
| H50 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| H51 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T | |
| H52 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | G | C | - | - | - | - | T | |

续表 2 Continued Table 2

| 单倍型 Hap | 简约信息位点 Parsimony informative site | | | | | | | | | | | | | | | | | | | | | | | | |
|------------|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | trnL-trnF | | | | | | | | rpl16 | | | | | | | | | | | | | | | | |
| | 230 | 355 | 414 | 456 | 611 | 792 | 813 | 928 | 966 | 988 | 1000 | 1090 | 1124 | 1192 | 1334 | 1352 | 1359 | 1360 | 1414 | 1451 | 1457 | 1465 | 1479 | 1551 | 1562 |
| H53 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H54 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H55 | - | - | - | - | - | - | - | - | - | C | - | - | A | - | - | - | - | - | - | - | - | - | - | - | - |
| H56 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | G | C | - | - | - | - | - | - | T |
| H57 | - | - | - | - | - | - | - | - | - | - | - | T | - | A | - | - | - | - | - | - | A | - | - | - | T |
| H58 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H59 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H60 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - |
| H61 | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H62 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H63 | - | - | - | - | - | - | - | - | - | C | - | - | A | - | - | - | - | - | - | - | - | - | - | - | - |
| H64 | - | - | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H65 | - | C | - | - | - | - | - | C | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H66 | - | - | - | - | C | - | - | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | G | T |
| H67 | - | - | - | - | - | - | - | - | T | - | - | T | - | - | - | - | - | - | - | A | A | - | A | - | T |
| H68 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H69 | - | C | - | - | - | - | A | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H70 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - |
| H71 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| H72 | A | - | - | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | T |
| H73 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - |
| H74 | - | - | - | - | - | - | - | - | - | - | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

注: -代表与单倍型 H_i 相同的碱基
 Note: - stands for the same base as haplotype H_i

表 3 唐古特虎耳草 18 个居群的单倍型组成、遗传多样性和核苷酸多样性

Table 3 Haplotype composition, gene diversity (*He*) and nucleotide diversity (π) of the 18 populations of *S. tangutica*

| 编号 Code | 单倍型组成(个体数) Haplotype composition (No. of individuals) | 遗传多样性 Gene diversity (<i>He</i>) | 核苷酸多样性 Nucleotide diversity (π) |
|------------|---|--|---|
| P1 | H1(2); H2(1); H3(5); H4(2); H5(2); H6(2) | 0.846 2 | 0.040 571 |
| P2 | H5 (5); H7 (1); H8 (1); H9 (4) | 0.709 1 | 0.007 506 |
| P3 | H2(4); H5(3); H7(3); H9(1); H10(4); H11(3); H12(1); H13(1); H14(2) | 0.904 8 | 0.017 659 |
| P4 | H5(4); H10(3); H15(1); H16(2) | 0.777 8 | 0.012 825 |
| P5 | H5(1); H17(2); H18(2); H19(1) | 0.866 7 | 0.012 071 |
| P6 | H1(1); H2(1); H8(2); H17(1); H20(1); H21(1); H22(1); H23(3); H24(1); H25(1); H26(1); H27(2); H28(1); H29(1); H30(2) | 0.968 4 | 0.042 394 |
| P7 | H5(3); H29(1); H30(1); H31(1) | 0.800 0 | 0.046 481 |
| P8 | H18(1); H32(1); H33(2); H34(1); H35(1) | 0.933 3 | 0.016 031 |
| P9 | H2(1); H5(5); H8(1); H36(1); H37(3); H38(1); H39(1) | 0.833 3 | 0.019 645 |
| P10 | H5(2); H40(1) | 0.666 7 | 0.007 832 |
| P11 | H1(2); H9(1); H41(3); H42(3); H43(1) | 0.844 4 | 0.015 071 |
| P12 | H5(1); H44(1); H45(2); H46(2); H47(1); H48(1); H49(1); H50(1) | 0.955 6 | 0.043 026 |
| P13 | H5(1); H17(1) | 1.000 0 | 0.009 729 |
| P14 | H5(1); H16(2); H20(1); H27(1); H30(2); H51(1) | 0.928 6 | 0.019 644 |
| P15 | H2(2); H35(1); H42(1); H43(1); H52(1); H53(1); H54(1); H55(1); H56(1); H57(1); H58(1); H59(1); H60(1); H61(1) | 0.990 5 | 0.024 551 |
| P16 | H5(8); H16(1); H29(1); H62(1) | 0.490 9 | 0.016 436 |
| P17 | H5(6); H9(4); H17(1); H30(1); H42(2); H46(1); H49(1); H58(2); H63(1); H64(1); H65(2); H66(1); H67(1) | 0.913 0 | 0.023 734 |
| P18 | H1(3); H3(1); H5(4); H43(1); H58(1); H68(1); H69(1); H70(1); 71(1); H72(2); H73(1); H74(1) | 0.934 6 | 0.023 409 |
| 平均 Mean | | 0.853 6 | 0.022 145 |

表 4 唐古特虎耳草 18 个居群的分子遗传变异 (AMOVA) 分析结果

Table 4 Analysis of molecular variance (AMOVA) for the 18 populations of *S. tangutica*

| 变异来源 Source of variation | 自由度 Degrees of freedom | 总方差 Sum of squares | 变异成分 Variation component | 变异比例 Percentage of variation / % |
|----------------------------------|---------------------------|-----------------------|-------------------------------|-------------------------------------|
| 居群间 Among populations | 17 | 73.560 | 0.190 65Va | 8.15 |
| 居群内 Within population | 191 | 410.651 | 2.150 00Vb | 91.85 |
| 总计 Total | 208 | 484.211 | 2.340 65 | |
| 固定系数 Fixation index (F_{ST}) | | | $F_{ST} = 0.08145 (P < 0.01)$ | |

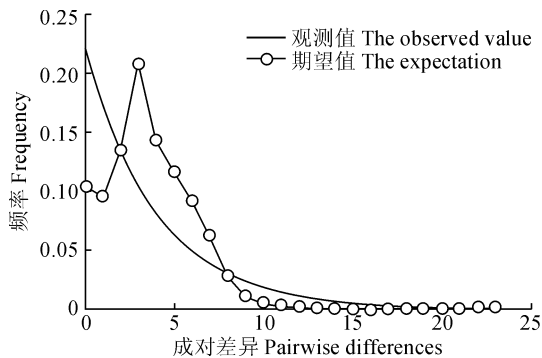


图 2 唐古特虎耳草 209 个个体叶绿体 DNA 2 个片段联合数据的歧点分布分析

Fig. 2 Mismatch distribution for 209 individuals of *S. tangutica* based on two cpDNA fragments

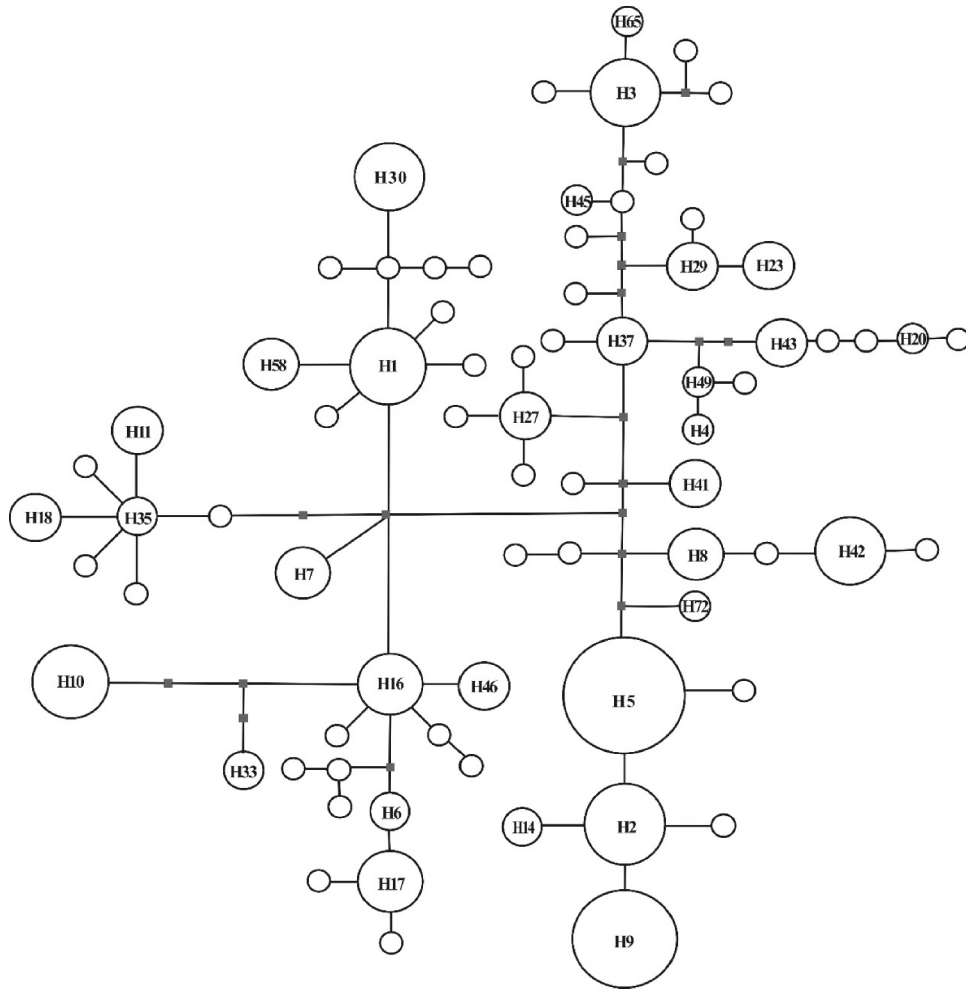
似然法 (maximum likelihood, ML)、贝叶斯法 (Bayesian inference, BI) 构建的单倍型系统发育树均呈现“刷子”结构,不能很好地揭示单倍型之间的系统发育关系,也表明了唐古特虎耳草单倍型的快速辐射分化(结果未显示)。NETWORK 软件可更好揭示遗传分化较浅序列之间的系统发育关系。基于最大似然法构建 74 个单倍型的中央链接网状图,结果显示(图 3),居群共享单倍型,如 H1、H2、H3、H5、H16 等位于网状图的中央位置,而居群特有单倍型一般位于网状图的边缘。根据溯祖理论,共享单倍型为较古老的单倍型,而居群特有单倍型可能是通过近期辐射分化衍生而来的较年轻的单倍型。

3 讨论

3.1 唐古特虎耳草的居群遗传结构

遗传变异分析表明唐古特虎耳草 cpDNA 片段总遗传多样性水平较高, $H_T = 0.933$ 。类似的结果也在青藏高原很多高山植物中被检测到,如偏花报春 (*Primula secundi flora*, $H_T = 0.966$)^[47]、西藏沙棘 (*Hippophae tibetana*, $H_T = 0.956$)^[26]、绵参 (*Eriophyton wallichii*, $H_T = 0.979$)^[48]、西川红景天 (*Rhodiola alsia*, $H_T = 0.950$)^[24]、菊叶红景天 (*Rhodiola chrysanthemifolia*, $H_T = 0.937$)^[27] 等。

然而,这些物种大多数都具有低的居群内平均遗传多样性。在本研究中,唐古特虎耳草不仅具有较高的总遗传多样性,居群内的平均遗传多样性也很高 ($H_S = 0.843$),表明唐古特虎耳草较高的遗传变异主要由居群内遗传变异所致。这一结果也得到了分子变异分析 (AMOVA) 的证实。AMOVA 分析表明,唐古特虎耳草居群内遗传变异占总变异的 91.85%,而居群间遗传变异仅为 8.15%,进一步揭示了唐古特虎耳草遗传变异主要存在于居群内。如此高的居群内遗传变异在青藏高原的植物中并不多见,推测造成该遗传结构的原因如下:一方面,由于检测到的 74 个单倍型中只有少数共享单倍型,但其个体数所占比例很高 (67.46%),并且这些共享单倍型在居群中随机分布,因此降低了居群间的遗传差异;另一方面,居群特有单倍型比例高 (53/74),某些居群固定多个特有单倍型,从而增加了居群内的遗传变异。特有单倍型比例较高的物种往往不能揭示出明显的谱系地理格局^[22, 24, 27]。此外,对虎耳草属山羊臭组 (*Saxifraga* sect. *Ciliatae* Haw.) 系统发育和物种形成的研究表明,唐古特虎耳草所在的唐古拉亚组 (subsect. *Hirculoideae* Engl. & Irm-sch.) 具有较快的分化速率,该亚组约 110 个物种是在最近的 2.12 百万年 (Ma) 分化而来^[10]。由此推测,唐古特虎耳草具有更短的居群分化历史。事实上,基于 NETWORK 构建的单倍型系统发育关系表明,大量的特有单倍型位于 NETWORK 图的边缘,揭示了唐古特虎耳草单倍型的近期快速分化。在第四纪冰期前,唐古特虎耳草可能广泛分布于青藏高原及其周边地区,并广泛固定某些古老单倍型 (如 H5)。随着第四纪冰期气候的波动,原来连续的居群片段化,形成了相互隔离的居群,并经历了异域分化,产生大量特有单倍型。在此过程中,瓶颈效应和遗传漂变等作用使得特有单倍型在居群中固定下来,抹去了居群原有的部分遗传结构。由于单独进化时间较短,特有单倍型之间的差异大多表现在



圆的大小表示单倍型频率,实心方块代表缺失单倍型

图 3 唐古特虎耳草 cpDNA 联合片段 74 种单倍型的中央连接网状图

Circle size is proportional to haplotype frequencies, parallelograms represent missing haplotypes

Fig. 3 Median-joining network of 74 haplotypes of *S. tangutica* based on two cpDNA fragments

单个碱基的变异。

3.2 冰期避难所及居群进化历史的推测

第四纪循环的气候波动对生物区系的分布和遗传结构产生了巨大的影响^[49]。植物对第四纪冰期的反应可以根据现有的遗传结构进行追溯。于海彬等^[50]综述了 36 种高山植物的谱系地理格局,总结出高山植物面对青藏高原复杂的地理结构、微气候和生境条件所呈现出的谱系历史。一部分高山植物在第四纪冰期时退缩到青藏高原边缘避难所,冰期后回迁到高原台面。符合这种“退缩-回迁”模式的物种,其遗传结构表现为遗传多样性较高的居群和古老单倍型往往集中在高原边缘避难所内,居群遗传多样性由高原边缘向高原台面递减。此外,一些耐寒植物,在冰期时没有完全退缩到低海拔的边缘地区,而是在高原台面形成了一个或几个相互隔离的避难所,冰期后发生局部扩张。符合这种“高原台

面避难所-局部扩张”模式的物种,其遗传结构表现为特有单倍型或者遗传多样性较高的居群分布在高原台面上的一个或几个相互隔离的区域。另外,某些物种第四纪冰期时在高原台面形成多个微型避难所,间冰期或冰期后发生小范围的扩张,其遗传多样性较高的居群和特有单倍型在物种的分布范围内均匀分布。

本研究没有揭示出唐古特虎耳草遗传多样性较高的某一地理区域,也没有发现遗传多样性由高原边缘向高原台面递减。相反,本研究所涉及的唐古特虎耳草居群普遍具有较高的遗传多样性,特有单倍型在分布范围内均匀分布。我们推测,由于青藏高原地形的高度异质性以及复杂的局部微气候和生境条件,在第四纪冰期时,唐古特虎耳草在高原台面上未被冰川覆盖的多个微型避难所中存活下来。唐古特虎耳草居群对第四纪冰期气候波动的反应可能

更多地表现为垂直海拔高度的迁移,而非大规模的水平迁移,这与银露梅(*Potentilla glabra*)^[22]和四川红景天(*Rhodiola chrysanthemifolia*)^[27]的进化历史相似。居群在垂直迁移的过程中发生了瓶颈效应和奠基者效应,居群随机固定少量古老单倍型。随后的异域分化和遗传漂变使得较年轻的衍生特有单倍型在居群中固定下来,形成了唐古特虎耳草现有的居群遗传结构。居群历史动态检验结果表明,Tajima's *D*和Fu & Li' *D**均为显著的负值,歧点分布分析呈单峰曲线,说明唐古特虎耳草经历过近期分布范围或者个体数的扩张^[51-53]。物种经历分布范围扩张事件,其原分布中心应具有较高的遗传多样性和特有单倍型,并且原分布中心的居群包含扩散区域居群所具有的单倍型。但在本研究中,大量的特有单倍型在唐古特虎耳草的整个分布范围内均匀分布,遗传多样性和核苷酸多样性较高的居群也呈现均匀分布,并未检测到分布范围扩张的信号。由此推测,该物种可能经历过个体数快速扩张事件,

从而导致了中性检验的负值和错配分布的单峰曲线。

综上所述,唐古特虎耳草具有较高的遗传变异,且主要由居群内遗传变异所致,大量特有单倍型是近期辐射分化的结果。唐古特虎耳草的进化历史与西藏圆柏复合群(*Juniperus tibetica* complex)^[25]、西藏沙棘(*Hippophae tibetana*)^[26]、菊叶红景天(*Rhodiola chrysanthemifolia*)^[27]相似,第四纪冰期时在高原台面存在多个微型避难所。第四纪冰期气候波动使得原来连续的居群片段化,形成了相互隔离的居群,并发生异域快速分化,形成大量特有单倍型。唐古特虎耳草居群对第四纪冰期气候波动的反应可能更多地表现为垂直海拔高度的迁移,而非大规模的水平迁移。此研究为青藏高原地区谱系地理学研究提供了一个新的案例,为了解第四纪冰期高山植物的避难所和现有分布格局的成因提供了参考依据。

参考文献:

- [1] MYERS N, MITTERMEIER R A, MITTERMEIER C G, *et al.* Biodiversity hotspots for conservation priorities [J]. *Nature*, 2000, **403**(6 772): 853-858.
- [2] HOORN C, MOSBRUGGER V, MULCH A, *et al.* Biodiversity from mountain building [J]. *Nature Geoscience*, 2013, **6**(3): 154.
- [3] HUGHES C E. The tropical Andean plant diversity powerhouse [J]. *New Phytologist*, 2016, **210**(4): 1 152-1 154.
- [4] FAVRE A, PÄCKERT M, PAULS S U, *et al.* The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas [J]. *Biological Reviews*, 2015, **90**(1): 236-253.
- [5] WEN J, ZHANG J-Q, NIE Z-L, *et al.* Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau [J]. *Frontiers in Genetics*, 2014, **5**: 4.
- [6] XING Y, REE R H. Uplift-driven diversification in the Hengduan Mountains, a temperate biodiversity hotspot [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2017, **114**(17): E3444-E3451.
- [7] EATON D A R, FENSTER C B, HEREFORD J, *et al.* Floral diversity and community structure in *Pedicularis* (Orobanchaceae) [J]. *Ecology*, 2012, **93**(8): S182-S194.
- [8] ZHANG J Q, MENG S Y, WEN J, *et al.* Phylogenetic relationships and character evolution of *Rhodiola* (Crassulaceae) based on nuclear ribosomal ITS and plastid *trnL-F* and *psbA-trnH* sequences [J]. *Systematic Botany*, 2014, **39**(2): 441-451.
- [9] WANG Y J, SUSANNA A, VON RAAB-STRAUBE E, *et al.* Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai-Tibetan Plateau [J]. *Botanical Journal of the Linnean Society*, 2009, **97**(4): 893-903.
- [10] GAO Q B, LI Y H, GORNALL R J, *et al.* Phylogeny and speciation in *Saxifraga* sect. *Ciliatae* (Saxifragaceae): evidence from *psbA-trnH*, *trnL-F* and ITS sequences [J]. *Taxon*, 2015, **64**(4): 703-713.
- [11] EBERSBACH J, MUELLNER-RIEHL A N, MICHALAK I, *et al.* In and out of the Qinghai-Tibet Plateau: divergence time estimation and historical biogeography of the large arctic-alpine genus *Saxifraga* L [J]. *Journal of Biogeography*, 2017, **44**(4): 900-910.
- [12] LIU J Q, WANG Y J, WANG A L, *et al.* Radiation and diversification within the *Ligularia-Cremathodium-Paraseneccio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau [J]. *Molecular Phylogenetics and Evolution*, 2006, **38**(1): 31-49.
- [13] ZHENG B, XU Q, SHEN Y. The relationship between climate change and Quaternary glacial cycles on the Qinghai-Ti-

- betan Plateau: review and speculation [J]. *Quaternary International*, 2002, **97-98**(1): 93-101.
- [14] OWEN L A, BENN D I. Equilibrium-line altitudes of the Last Glacial Maximum for the Himalaya and Tibet: an assessment and evaluation of results [J]. *Quaternary International*, 2005, **138-139**(3): 55-78.
- [15] LEHMKUHL F, OWEN L A. Late Quaternary glaciation of Tibet and the bordering mountains: a review [J]. *Boreas*, 2005, **34**(2): 87-100.
- [16] HEWITT G M. Post-glacial re-colonization of European biota [J]. *Biological Journal of the Linnean Society*, 1999, **68**(1/2): 87-112.
- [17] HEWITT G M. Speciation, hybrid zones and phylogeography-or seeing genes in space and time [J]. *Molecular Ecology*, 2001, **10**(3): 537-549.
- [18] ZHANG D, FENGQUAN L, JIANMIN B. Eco-environmental effects of the Qinghai-Tibet Plateau uplift during the Quaternary in China [J]. *Environmental Geology*, 2000, **39**(12): 1 352-1 358.
- [19] HEWITT G M. Genetic consequences of climatic oscillations in the Quaternary [J]. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 2004, **359**(1 442): 183-195.
- [20] ZHANG Q, CHIANG T Y, GEORGE M, *et al.* Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation [J]. *Molecular Ecology*, 2005, **14**(11): 3 513-3 524.
- [21] MENG L, YANG R, ABBOTT R J, *et al.* Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands [J]. *Molecular Ecology*, 2007, **16**(19): 4 128-4 137.
- [22] WANG L Y, IKEDA H, LIU T L, *et al.* Repeated range expansion and glacial endurance of *Potentilla glabra* (Rosaceae) in the Qinghai-Tibetan Plateau [J]. *Journal of Integrative Plant Biology*, 2009, **51**(7): 698-706.
- [23] WANG L, ABBOTT R J, ZHENG W, *et al.* History and evolution of alpine plants endemic to the Qinghai-Tibetan Plateau: *Aconitum gymmandrum* (Ranunculaceae) [J]. *Molecular Ecology*, 2009, **18**(4): 709-721.
- [24] GAO Q, ZHANG D, DUAN Y, *et al.* Intraspecific divergences of *Rhodiola alsia* (Crassulaceae) based on plastid DNA and internal transcribed spacer fragments [J]. *Botanical Journal of the Linnean Society*, 2012, **168**(2): 204-215.
- [25] OPGENOORTH L, VENDRAMIN G G, MAO K, *et al.* Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the Last Glacial Maximum [J]. *New Phytologist*, 2010, **185**(1): 332-342.
- [26] WANG H, QIONG L, SUN K, *et al.* Phylogeographic structure of *Hippophae tibetana* (Elaeagnaceae) highlights the highest microrefugia and the rapid uplift of the Qinghai-Tibetan Plateau [J]. *Molecular Ecology*, 2010, **19**(14): 2 964-2 979.
- [27] GAO Q B, ZHANG F Q, XING R, *et al.* Phylogeographic study revealed microrefugia for an endemic species on the Qinghai-Tibetan Plateau: *Rhodiola chrysanthemifolia* (Crassulaceae) [J]. *Plant Systematics and Evolution*, 2016, **302**(9): 1 179-1 193.
- [28] WU C Y, RAVEN P H. Flora of China [M]. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press, 2001, **8**: 208-344.
- [29] ABBOTT R J, COMES H P. Evolution in the Arctic: a phylogeographic analysis of the circumarctic plant, *Saxifraga oppositifolia* (Purple saxifrage) [J]. *New Phytologist*, 2003, **161**(1): 211-224.
- [30] DECHAINE E G, ANDERSON S A, MCNEW J M, *et al.* On the evolutionary and biogeographic history of *Saxifraga* sect. *Trachyphyllum* (Gaud.) Koch (Saxifragaceae Juss.) [J]. *PLoS One*, 2013, **8**(7): e69814.
- [31] EBERSBACH J, SCHNITZLER J, FAVRE A, *et al.* Evolutionary radiations in the species-rich mountain genus *Saxifraga* L [J]. *BMC Evolutionary Biology*, 2017, **17**(1): 119.
- [32] GAO Q B, LI Y, GENGJI Z M, *et al.* Population genetic differentiation and taxonomy of three closely related species of *Saxifraga* (Saxifragaceae) from southern Tibet and the Hengduan Mountains [J]. *Frontiers in Plant Science*, 2017, **8**: 1 325.
- [33] DOYLE J J, DOYLE J L. A rapid DNA isolation procedure for small quantities of fresh leaf material [J]. *Phytochemical Bulletin, Botanical Society of America*, 1987, **19**(1): 11-15.
- [34] TABERLET P, GIELLY L, PAUTOU G, *et al.* Universal primers for amplification of three non-coding regions of chloroplast [J]. *Plant Molecular Biology*, 1991, **17**(5): 1 105-1 109.
- [35] SCARCELLI N, BARNAUD A, EISERHARDT W, *et al.* A set of 100 chloroplast DNA primer pairs to study population genetics and phylogeny in Monocotyledons [J]. *PLoS One*, 2011, **6**(5): e19954.
- [36] KUMAR S, STECHER G, TAMURA K. MEGA 7: Molecular evolutionary genetics analysis version 7. 0 for bigger

- datasets [J]. *Molecular Biology and Evolution*, 2016, **33**(7): 1 870-1 874.
- [37] LIBRADO P, ROZAS J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data [J]. *Bioinformatics*, 2009, **25**(11): 1 451-1 452.
- [38] PONS O, PETIT R J. Measuring and testing genetic differentiation with ordered versus unordered alleles [J]. *Genetics*, 1996, **144**(3): 1 237-1 245.
- [39] EXCOFFIER L, LISCHER H E L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows [J]. *Molecular Ecology Resources*, 2010, **10**(3): 564-567.
- [40] WEIR B S, COCKERHAM C C. Estimating F-Statistics for the analysis of population structure [J]. *Evolution*, 1984, **38**(6): 1 358-1 370.
- [41] TAJIMA F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism [J]. *Genetics*, 1989, **123**(3): 585-595.
- [42] FU Y X, LI W H. Statistical tests of neutrality of mutations [J]. *Genetics*, 1993, **133**(3): 693-709.
- [43] BANDELT H J, FORSTER P, RÖHL A. Median-joining networks for inferring intraspecific phylogenies [J]. *Molecular Biology and Evolution*, 1999, **16**(1): 37-48.
- [44] POLZIN T, DANESHMAND S V. On Steiner trees and minimum spanning trees in hypergraphs [J]. *Operations Research Letters*, 2003, **31**(1): 12-20.
- [45] GOLDING G B. The detection of deleterious selection using ancestors inferred from a phylogenetic history [J]. *Genetics Research*, 1987, **49**(1): 71-82.
- [46] CRANDALL K A, TEMPLETON A R. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction [J]. *Genetics*, 1993, **134**(3): 959-969.
- [47] WANG F Y, GONG X, HU C M, *et al.* Phylogeography of an alpine species *Primula secundiflora* inferred from the chloroplast DNA sequence variation [J]. *Journal of Systematics and Evolution*, 2008, **46**(1): 13-22.
- [48] 王晓雄, 乐霖培, 孙航, 等. 青藏高原高山流石滩特有植物绵参的谱系地理学研究 [J]. *植物分类与资源学报*, 2011, **33**(6): 605-614.
- WANG X X, YUE J P, SUN H, *et al.* Phylogeographical study on *Eriophyton wallichii* (Labiatae) from Alpine scree of Qinghai-Tibetan Plateau [J]. *Plant Diversity & Resources*, 2011, **33**(6): 605-614.
- [49] DYNESIUS M, JANSSON R. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2000, **97**(16): 9 115-9 120.
- [50] 于海彬, 张镜铨. 青藏高原及其周边地区高山植物谱系地理学研究进展 [J]. *西北植物学报*, 2013, **33**(6): 1 268-1 278.
- YU H B, ZHANG Y L. Advances in phylogeography of alpine plants in the Tibetan Plateau and adjacent regions [J]. *Acta Botanica Boreali-Occidentalia Sinica*, 2013, **33**(6): 1 268-1 278.
- [51] RAY N, CURRAT M, EXCOFFIER L. Intra-deme molecular diversity in spatially expanding populations [J]. *Molecular Biology and Evolution*, 2003, **20**(1): 76-86.
- [52] EXCOFFIER L. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model [J]. *Molecular Ecology*, 2004, **13**(4): 853-864.
- [53] HARPENDING H C, BATZER M A, GURVEN M, *et al.* Genetic traces of ancient demography [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1998, **95**(4): 1 961-1 967.

(编辑:潘新社)