Diploid and tetraploid distribution of *Allium przewalskianum* Regel. (Liliaceae) in the Qinghai-Tibetan Plateau and adjacent regions

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Abstract — Polyploidization plays an important role in generating the current high diversity of plants. Studies of distributional patterns of diploids and derivative autopolyploids have provided important insights into evolutionary processes and cryptic speciation of polyploidization within species defined on the basis of their morphology. However, few studies have been designed to examine distributions of infrageneric diploids and polyploids on the Asian Qinghai-Tibetan Plateau (QTP). *Allium przewalskianum* occurs widely on the QTP and in adjacent regions, at altitudes ranging from 2000m to 4500m. We collected a total of 844 individuals from 62 populations and determined their cytotypes over the entire distribution range of this species. Tetraploids tend to occur at high altitudes; however, the positive relationship between the ploidy and altitude was only marginally significant ($P < 0.05$). Contact zones between diploids and tetraploids were recorded on the eastern QTP from north to south. Four populations were found to harbor both cytotypes, but no triploid individuals. The wider distribution of tetraploids may be mainly due to their greater colonization ability in the new niches created by the Quaternary climatic oscillations in the QTP region. Our results offer a fundamental framework for studying evolutionary origins, adaptations and cryptic divergences of polyploids within this species complex based on molecular and/or ecological examinations in the future.

Key words: *Allium przewalskianum*; altitude; colonization; contact zones; cryptic speciation; polyploidy

INTRODUCTION

Polyploid plants are very common (Stebbins 1971; Grant 1981) and the occurrence of polyploidy in angiosperm species has been estimated to range from 57% to 70%, based solely on chromosome numbers among extant species (Goldblatt 1980; Masterson 1994; Leitch and Bennett 1997). Recent studies have suggested that polyploidy has played a much more important role in plant evolution and speciation (leading to the current biodiversity) than previously believed; a few species that were assumed to be diploids (for example, the model species *Arabidopsis thaliana*) have been found to have experienced one or more episodes of polyploidy in their evolutionary past (Levin 2002).

The common occurrence of polyploidy can also be seen from the long-known diploid-autopolyploid complexes: different populations (individuals) within a single morphological species have different chromosomal cytotypes (Grant 1981). These intraspecific cytotypes are usually maintained as "cryptic species" due to reproductive isolation, but could not be identified by morphological variations (Levin 2002). Furthermore, autopolyploids have been shown to have recurrent origins in many species investigated, providing further evidence for frequent polyploidizations and complex speciation process during the evolution of plants (Bever and Felber 1992; Rodriguez 1996; Husband and Schemske 1998; McArthur and Sanderson 1999; Stuessy *et al.* 2004). However, due to the limited number of species studied, the mechanisms associated with the origins of autopolyploids, their initial establishment and coexistence with the diploids, and whether multiple origins are typical, remain largely unknown. In addition, the

evolutionary advantages of autopolyploidy are still debated. Autopolyploidy was once considered to result in evolutionary dead ends (but see SOLTIS and RIESEBERG 1986). The frequent occurrences of autopolyploids were thought, by a few researchers, to be mainly the result of historical selection of the dominant polyploid genotypes during recolonization after the Last Glacial Maximum (e.g. van DIJK and BAKX-SCHOTMAN 1997). Others, however, have attributed successful colonization by autopolyploids to their improved adaptive abilities (SOLTIS and SOLTIS 2000; LEVIN 2002). For example, polysomic inheritance can maintain a high level of heterozygosity, and therefore may reduce inbreeding depression and enhance the colonization ability of autopolyploids.

A detailed investigation of the distribution of diploids and derivative autopolyploids can provide critical insights into the origins and establishment of new polyploids and cryptic speciation within a morphological species (SEGRAVES et al. 1999; BAACK 2004; STUESSY et al. 2004). In addition, such a distributional study can answer a series of questions regarding the evolution of autopolyploidy and speciation, i.e., niche differentiation, barriers to coexistence, adaptation fitness and colonization ability in arid habitats (van DIJK and BAKX-SCHOTMAN 1997; HUSBAND 2000). The intraspecific autopolyploids as well diploids have been widely recorded for plants occurring in the arctic regions, European alpine mountains and North American temperate regions (TISCHLER 1935; LÖVE and LÖVE 1949; EHRENDORFER 1980; GRANT 1981; STEBBINS 1984; 1985; STEBBINS and DAWE 1987; SEGRAVES et al. 1999; BAACK 2004; BROCHMANN et al. 2004; STUESSY et al. 2004; BESNARD et al. 2008; MRÁZ et al. 2008). However, few studies have been intentionally designed to examine occurrences of intraspecific autopolyploids at the regional scale along the ecological gradients. The Qinghai-Tibetan Plateau (QTP) is the highest and largest plateau in the world, with an average elevation of 4000 m above sea level; the biota occurring there are considered to be highly sensitive and vulnerable to global climate change because their growth and distribution in the region depend greatly on survivable temperatures (ZHENG 1996). Extensive research has revealed that most species in this region have experienced glacial retreat and postglacial recolonization at high altitudes (see MENG et al. 2007; ZHANG et al. 2005). In the eastern QTP, altitudes vary greatly and ecological niches are extremely diverse (WU and WU 1996). All these factors may have contributed to the origins of plant neopolyploids, and the diploid-polyploid species complexes in this region may provide important models for examining the evolutionary processes and cryptic speciation associated with polyploids and niche differentiation along the altitudinal gradients.

Allium is a large genus within the Liliaceae, with more than 400 species widely distributed throughout the temperate Northern Hemisphere (HANELT et al. 1992). This genus exhibits great variation in its chromosomal numbers at all taxonomical levels. The basic chromosome numbers range from x = 7, to x = 8, 10 and 11 and these different numbers have been employed to circumscribe subgenera and sections of this genus, and explain the historical phylogeny of these groups. The phylogenetic relationships inferred from these chromosomal differences have be supported by morphological characters of the leaves and scape, and by recent molecular phylogeny analyses (SAMOYLOV et al. 1995; LINNE VON BERG et al. 1996; MES et al. 1998; SAMOYLOV et al. 1999). At the species level, the variation in levels of ploidy and karyotypic structures are common both between and within species. These chromosomal characteristics, as well as geographical information, have also been used to elucidate the origin of species and to interpret their roles in speciation and reproductive isolation (SUNDBERG and STUESSY 1990). Intraspecific autotetraploids prevail in this genus and only a few of them have been examined in detail to confirm their polysomic inheritance (RIESEBERG and DOYLE 1989).

Allium przewalskianum is widely distributed across the QTP and in adjacent low altitude regions, where it grows in scrub, on dry slopes, on plains and in rock crevices at elevations between 2000 m and 4500 m. This species is morphologically and genetically distinct from all the closely related species within the section *Rhizirideum* of the subgenus *Rhizirideum* (ZHOU et al. 2006). A previous investigation of cytotypes of nine populations of this species in Qinghai suggested that both diploids and tetraploids co-occur, and that it is a typical diploid-polyploid species complex (XUE et al. 2000). In this study, we conducted an exhaustive survey of the chromosomal races in most populations within the natural range of *A. przewalskianum* across the QTP and the adjacent regions. The specific goals of this study were to: (1) investigate in detail the distribution of diploids and polyploids within the natural range of *A. przewalskianum*; (2) characterize their contact zones and (3) examine whether diploid and tetraploid populations show segregation in relation to each other along the altitudinal gradients.

MATERIALS AND METHODS

The sampled populations are listed in Table 1 and their locations shown in Fig. 1. The collection of the materials in the QTP continued for five years from 2003 to 2007 and the corresponding voucher specimens were deposited in the Institute of Molecular Ecology, Lanzhou University or Northwest Plateau Institute of Biology, the Chinese Academy of Sciences. In each population, between 4 and 30 individuals were sampled, spaced at least 2 m apart depending on the sample size. A total of 844 individuals from 62 populations were collected. We collected fresh roots in two ways: the fresh roots of individual plants were either collected directly in the field or were harvested after individual plants had been transferred back to the greenhouse and cultivated for two weeks. Two to ten roots were collected from each individual. The root tips of each plant, to a length of 0.3cm, were excised and pretreated with a mixture of 1:1 (v/v) 8-hydroxyquinoline (0.002% w/v): colchicine (0.05% w/v) for 2-3h at 25°C, fixed and stored in Carnoy's solution (3 absolute ethanol: 1 glacial acetic acid), awaiting further analysis. Following fixation, they were macerated in 1N hydrochloric acid at 60°C for 6 min, then stained and squashed with Carbol Fuchsin. For each individual, at least 5 cells with well-spread chromosomes were used to determine the number of chromosomes. The correlation between altitude and ploidy was tested using Pearson Correlation Analysis by means of a linear regression model in the software package SSPS 13.0 (SPSS Inc., Chicago, IL). We used a similar method to test the regional segregation of cytotypes. We tested for independence of the two distributions using Fisher's exact test with one degree of freedom.

RESULTS

A total of 844 individuals, from 62 populations of *A. przewalskianum* (Table 1), were examined to determine chromosome number. The chromosomes of all sampled plants were either 2n = 16 or 2n = 32, with a few individuals possessing additional B chromosomes (Fig. 2). B-chromosomes
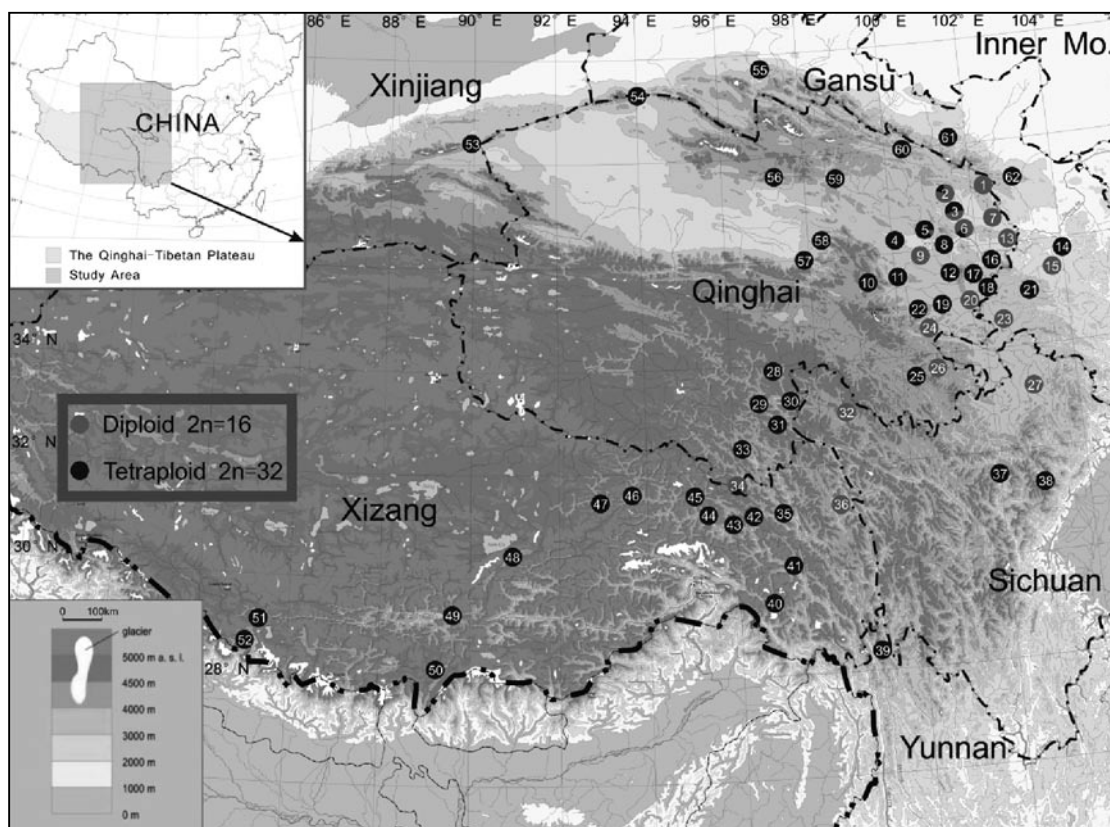

Fig. 1 — Distribution of the diploid and tetraploid cytotypes of *Allium przewalskianum* in the Qinghai-Tibetan Plateau and adjacent regions. The local details for each population are given in Table 1.
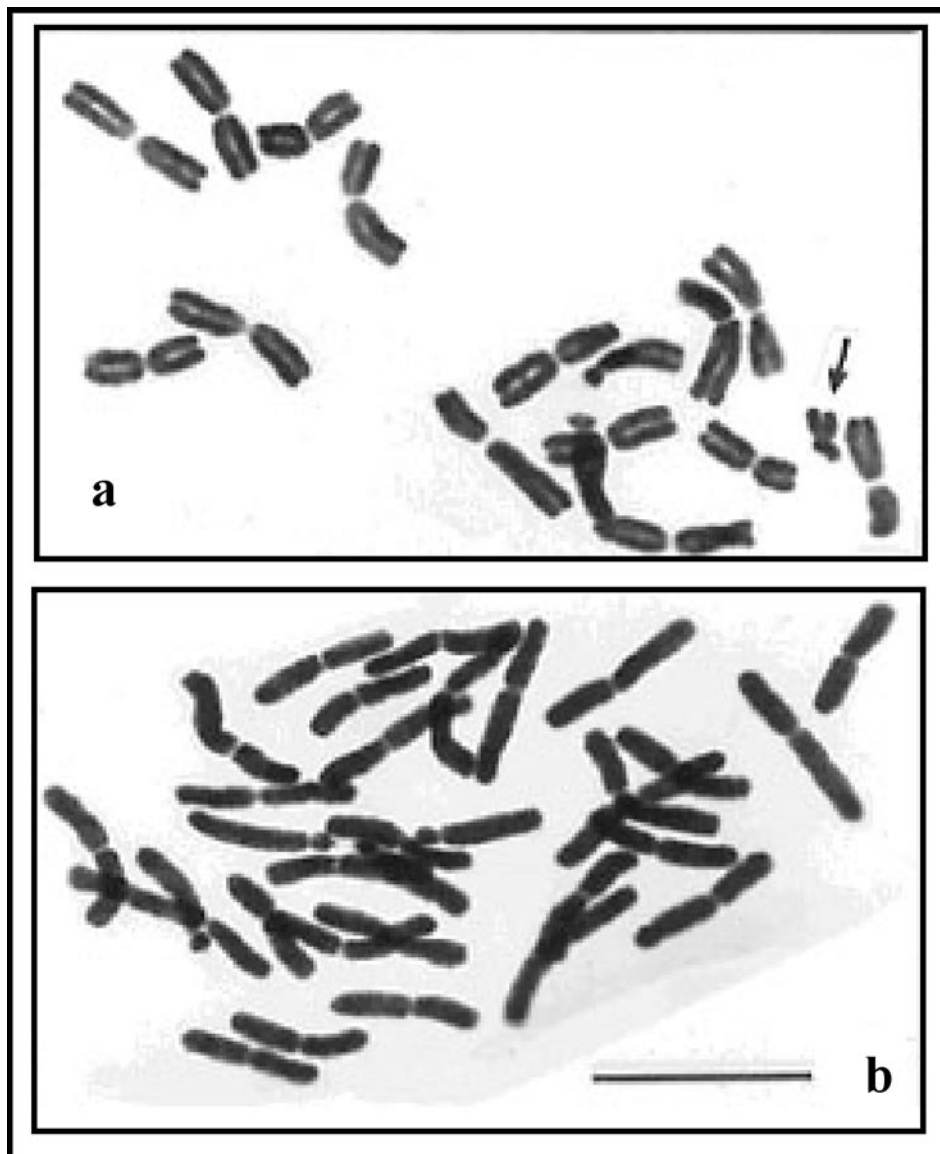
Fig. 2 — A diploid and a tetraploid *A. przewalskianum* a. Chromosomes (2n = 16) of one diploid individual from Liu-Ao population 117, the arrow indicates a B chromosome; b. Chromosomes (2n = 32) of one tetraploid individual from Liu-Ao population 78. Bar = 10 um.

were found in a few diploid individuals from four populations (9, 32, 34 and 36) (Table 1). These new counts, together with data from a previous report (XUE *et al.* 2000), confirm that the ploidy level in this species is diploid with n = 8 and tetraploid with n = 16. The diploid populations mainly occur in the east of the QTP while the tetraploid ones tend to grow at high altitudes in the west. The contact zone between them is wide, and ranges from the north (population 1) to the south (population 34) (Fig. 1). Four populations (2, 3, 6 and 34) were found to harbor both cytotypes; no triploid individuals were detected. The two different cytotypes have no distinct segregation structure; in the northeast region their distributions overlap. For example, the diploid population 9 was surrounded by tetraploid populations. In general, the tetraploids have a wider distribution than diploids and tend to occupy the high altitude regions. However, diploids also occur in sites with altitudes above 4000 m and the distributions overlapped in regions at altitudes between 2200 m and 2400 m (Fig. 3). We used Pearson Correlation Analysis to test the relationship between altitude and ploidy
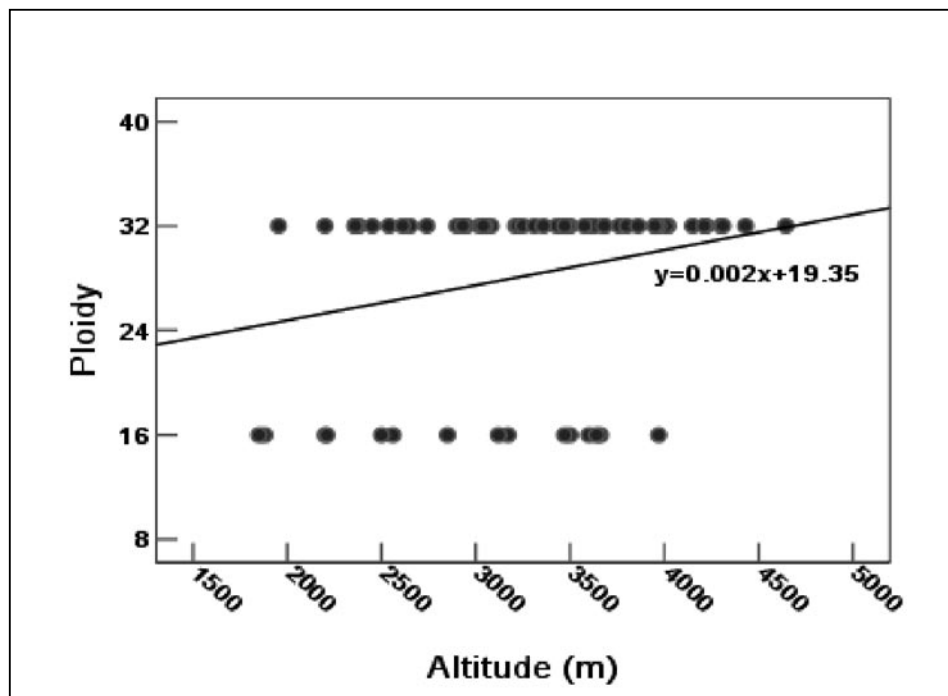
Fig. 3 — Scatter plot of ploidy (2n=16 and 2n=32) versus altitude (meters) of *A. przewalskianum* throughout its distributional range. The linear relationship indicates that the positive association between the ploidy and altitude was marginally significant ($r^2 = 0.072$, $P < 0.05$).

of the sampled populations. We found a low positive correlation, $r^2 = 0.072$ ($P < 0.05$), but this was only just significant. Diploid and tetraploid populations were distributed at random in relation to each other; they did not tend to cluster with others of the same cytotype ($P > 0.05$).

DISCUSSION

Our results suggest that within the total geographical range of *A. przewalskianum*, tetraploids have a wider distribution than diploids. In the high altitude region of the western QTP, only tetraploids were recovered. However, in the eastern region, diploids and tetraploids could not differentiated into two parapatric distributions ($P > 0.05$) with no mixing, nor did their distribution resemble a gradual, clinal transition from one cytotype to the other across altitudinal, latitudinal or longitudinal gradients as have been described for many hybrid zones (HARRISON and RAND 1989). In early studies, the frequencies and levels of polyploidy were found to increase with altitude and latitude (TISCHLER 1935). Most researchers, therefore, assumed that polyploids had higher fitness in arid conditions and more flexible adaptation to

climatic oscillations than diploids (LÖVE and LÖVE 1949; GRANT 1981; SOLTIS *et al.* 2003). However, this "increased hardiness hypothesis" was rejected by EHRENDORFER (1980), STEBBINS (1984; 1985) and STEBBINS and DAWE (1987). They suggested that polyploids tend to originate in climatically unstable conditions and are more successful than diploids in colonizing glaciated regions. This assumption was further supported by the latest review of arctic polyploids, presented by BROCHMANN *et al.* (2004). However, the distributional patterns of the two cytotypes of *A. przewalskianum* do not favor either of the two alternative hypotheses of the evolutionary processes associated with polyploids. The current distributions may result from a combination of the two hypotheses. On the one hand, most of the current range of the tetraploids is within an area of the high altitude QTP that was subject to serious climatic oscillations. This suggests that the wide distribution of these tetraploids is probably due to postglacial range expansion within the Holocene. In the west QTP (westward from populations 9, 32 and 34), all populations are tetraploid; this region was completely covered by deserts or alpine tundra during the Last Glacial Maximum (TANG *et al.* 1998). In contrast, in the

Table 1 — The population locations and chromosome numbers of Allium przewalskianum.

Population	Locality	Geographical position	Altitude	Chromosome number (the examined individuals)	Voucher specimens
1	Huzhu, Qinghai, China	N 37°03.029' E 102°23.934'	2378m	2n = 16 (12)	J. Q. Liu-AO 01
2	Haiyan, Qinghai, China	N 36°51.367' E 101°03.252'	2950m	2n = 16 (1) 2n = 32 (16)	J. Q. Liu-AO 14
3	Huangyuan, Qinghai, China	N 36°39.920' E 101°23.567'	2470-2520m	2n = 16 (10) 2n = 32 (12)	J. Q. Liu-AO 162
4	Gonghe, Qinghai, China	N 36°22.386' E 100°23.673'	3490m	2n = 32 (17)	J. Q. Liu-AO 80
5	Qinghai Lake, Qinghai, China	N 36°33.157' E 100°43.757'	3210m	2n = 32 (28) 2n = 16 (3)	J. Q. Liu-AO 78
6	Xining, Qinghai, China	N 36°33.786' E 101°54.361'	2210m	2n = 16 (15) 2n = 32 (2)	J. Q. Liu-AO 72
7	Ping'an, Qinghai, China	N 36°38.717' E 102°02.336'	2200m	2n = 16 (11)	J. Q. Liu-AO 70
8	Huangzhong, Qinghai, China	N 36°30.434' E 101°37.905'	2540m	2n = 32 (9)	J. Q. Liu-AO 75
9	Gonghe, Qinghai, China	N 35°57.803' E 100°59.769'	2850m	2n = 16 (9) 2n = 16+1B (1)	J. Q. Liu-AO 77
10	Xinghai, Qinghai, China	N 35°53.205' E 099°40.588'	3760m	2n = 32 (13)	J. Q. Liu-AO 85
11	Xinghai, Qinghai, China	N 35°44.433' E 100°13.405'	3220m	2n = 32 (17)	J. Q. Liu-AO 82
12	Tongren, Qinghai, China	N 35°22.337' E 101°37.747'	2740m	2n = 32 (14)	J. Q. Liu-AO 25
13	Ledu, Qinghai, China	N 36°25.007' E 102°46.160'	2560m	2n = 16 (10)	J. Q. Liu-AO 69
14	Yuzhong, Gansu, China	N 35°46.796' E 104°01.378'	2450m	2n = 32 (11)	J. Q. Liu-AO 67
15	Lintao, Gansu, China	N 35°23.327' E 103°50.402'	1880m	2n = 16 (11)	J. Q. Liu-AO 66
16	Tongren, Qinghai, China	N 35°42.662' E 102°17.652'	3020m	2n = 32 (5)	J. Q. Liu-AO 23
17	Tongren, Qinghai, China	N 35°35.598' E 102°03.158'	2360m	2n = 32 (21)	J. Q. Liu-AO 56
18	Tongren, Qinghai, China	N 35°26.772' E 102°27.402'	3080m	2n = 32 (15)	J. Q. Liu-AO 62
19	Zeku, Qinghai, China	N 35°16.497' E 101°06.670'	3500m	2n = 32 (18)	J. Q. Liu-AO 41
20	Tongren, Qinghai, China	N 35°15.775' E 101°52.997'	3170m	2n = 16 (9)	J. Q. Liu-AO 54
21	Hezuo, Gansu, China	N 35°08.350' E 102°51.059'	2650m	2n = 32 (9)	J. Q. Liu-2006039
22	Ningxiu, Qinghai, China	N 35°07.752' E 100°43.657'	3450m	2n = 32 (15)	J. Q. Liu-AO 43
23	Luqu, Gansu, China	N 34°35.576' E 102°28.045'	3120m	2n = 16 (19)	J. Q. Liu-2006033
24	Henan, Qinghai, China	N 34°44.195' E 101°18.090'	3600m	2n = 16 (12)	J. Q. Liu-AO 30
25	Maqing, Qinghai, China	N 33°34.843' E 100°33.333'	3430m	2n = 32 (21)	J. Q. Liu-AO 52
26	Guoluo, Qinghai, China	N 33°43.289' E 100°48.921'	3500m	2n = 16 (19)	J. Q. Liu-AO 53
27	Hongyuan, Sichuan, China	N 33°00.880' E 102°36.397'	3470m	2n = 16 (7)	J. Q. Liu-2006026
28	Yushu, Qinghai, China	N 33°50.522' E 097°10.601'	4020m	2n = 32 (13)	J. Q. Liu-AO 89
29	Yushu, Qinghai, China	N 32°51.782' E 096°57.465'	3950m	2n = 32 (15)	J. Q. Liu-AO 106
30	Yushu, Qinghai, China	N 33°11.008' E 097°23.636'	4010m	2n = 32 (28)	J. Q. Liu-AO 92
31	Yushu, Qinghai, China	N 33°00.198' E 097°19.007'	3610m	2n = 32 (26)	J. Q. Liu-AO 95
32	Shiqu, Sichuan, China	N 32°59.862' E 098°20.369'	3970m	2n = 16 (24) 2n = 16+1B (2)	J. Q. Liu-AO 108
33	Yushu, Qinghai, China	N 32°20.524' E 096°25.850'	3680m	2n = 32 (22)	J. Q. Liu-AO 101
34	Yushu, Qinghai, China	N 31°51.329' E 096°17.741'	3660m	2n = 32 (4) 2n = 16 (30) 2n = 16+1B (3)	J. Q. Liu-AO 103
35	Changdu, Xizang, China	N 31°08.853' E 097°01.170'	3630m	2n = 32 (32)	J. Q. Liu-AO 119
36	Jiangda, Xizang, China	N 31°26.607' E 098°09.073'	3640m	2n = 16+1B (12) 2n = 16+2B (2)	J. Q. Liu-AO 117
37	Jinchuan, Sichuan, China	N 31°27.843' E 102°05.967'	2150-2280m	2n = 32 (7)	J. Q. Liu-2006018
38	Lixian, Sichuan, China	N 31°27.874' E 103°06.922'	1954m	2n = 32 (5)	J. Q. Liu-TB07024
39	Deqin, Yunan, China	N 28°27.626' E 098°54.589'	3040m	2n = 32 (4)	J. Q. Liu-2006310
40	Ranwu, Xizang, China	N 29°30.292' E 096°45.802'	3950m	2n = 32 (4)	J. Q. Liu-2006290
41	Basu, Xizang, China	N 30°11.523' E 097°19.351'	4210m	2n = 32 (5)	J. Q. Liu-2006297
42	Leiwuqi, Xizang, China	N 31°12.321' E 096°36.056'	3800m	2n = 32 (19)	J. Q. Liu-AO 121
43	Leiwuqi, Xizang, China	N 30°57.976' E 096°14.537'	4150m	2n = 32 (6)	J. Q. Liu-AO 126
44	Dingqing, Xizang, China	N 31°18.364' E 095°45.659'	3680m	2n = 32 (7)	J. Q. Liu-AO 128
45	Dingqing, Xizang, China	N 31°41.341' E 095°01.492'	3860m	2n = 32 (20)	J. Q. Liu-AO 130
46	Baqing, Xizang, China	N 31°48.803' E 093°44.462'	3970m	2n = 32 (19)	J. Q. Liu-AO 132
47	Naqu, Xizang, China	N 31°45.293' E 092°46.547'	4220m	2n = 32 (12)	J. Q. Liu-AO 134
48	Dangxiong, Xizang, China	N 30°32.355' E 091°07.669'	4310m	2n = 32 (4)	J. Q. Liu-AO 157
49	Jiangzi, Xizang, China	N 28°50.995' E 089°55.043'	4300m	2n = 32 (18)	J. Q. Liu-AO 142
50	Kangma, Xizang, China	N 28°32.649' E 089°42.990'	4430m	2n = 32 (8)	J. Q. Liu-2006219
51	Jilong, Xizang, China	N 28°48.806' E 085°30.074'	4644m	2n = 32 (10)	J. Q. Liu-2007140
52	Jilong, Xizang, China	N 28°26.737' E 085°14.864'	3026-4126m	2n = 32 (10)	J. Q. Liu-2007042
53	Aerjinshan, Gansu, China	N 38°33.254' E 090°10.182'	3358m	2n = 32 (8)	J. Q. Liu-TB07121
54	Dangjinshan, Gansu, China	N 39°22.601' E 094°19.511'	2930m	2n = 32 (8)	J. Q. Liu-TB07117
55	Yumen, Gansu, China	N 39°48.429' E 097°17.189'	2361m	2n = 32 (5)	J. Q. Liu-TB07113
56	Delingha, Qinghai, China	N 37°26.826' E 097°20.282'	3310m	2n = 32 (9)	J. Q. Liu-AO 160
57	Dulan, Qinghai, China	N 36°00.821' E 097°53.443'	3050m	2n = 32 (2)	J. Q. Liu-AO 158
58	Dulan, Qinghai, China	N 36°14.677' E 098°06.370'	3250m	2n = 32 (15)	J. Q. Liu-AO 159
59	Wulan, Qinghai, China	N 37°04.310' E 098°52.464'	3470m	2n = 32 (8)	J. Q. Liu-AO 161
60	Ebo, Qinghai, China	N 37°59.878' E 100°45.921'	3223m	2n = 32 (15)	J. Q. Liu-AO 03
61	Shandan, Gansu, China	N 38°06.326' E 101°33.209'	2612m	2n = 32 (6)	J. Q. Liu-TB07101
62	Wuwei, Gansu, China	N 37°12.218' E 102°51.436'	2900m	2n = 32 (5)	J. Q. Liu-TB07099

eastern QTP, steppes and sparse forest patches may have persisted during this period. It is highly likely that tetraploids originated during the last glacial period when climates oscillated extensively in this region, and then colonized the west QTP when the temperature increased again and new niches appeared. On the other hand, the greater postglacial colonization ability of tetraploids could not explain the current distribution of the two cytotypes in the eastern QTP. The ancestral diploids should have persisted in this region during the glacial stages and probably colonized most regions at the end of the glaciations. The initial development of tetraploids then gradually replaced a few diploids at high altitudes within the Holocene mainly because of their increased fitness. Although unconfirmed, these ideas offer a framework for future studies based on molecular data. Clearly, reciprocal transplant experiments are also necessary in order to confirm the suggested greater fitness of tetraploids.

The contact zone of the two cytotypes of *A. przewalskianum* extended for more than 400 kilometers from north to south (Fig. 1). The mosaic pattern of the two intermixed cytotypes of *A. przewalskianum* in the eastern QTP contrasts with the findings associated with most allo- and autopolyploid taxa, where diploids and tetraploids are geographically separated over most of their distributions (CLAUSEN et al. 1945; STEBBINS and ZOHARY 1959; KAY 1969; SOLTIS 1984; ROTHERA and DAVY 1986; LUMARET et al. 1987; FELBER 1988; van DIJK et al. 1992; HUSBAND and SCHEMSKE 1998; BAACK 2004; STUESSY et al. 2004). It should be noted, exceptions, however, do occur (e.g., NESOM 1983; KAO 2008). The contact zones can be the result of two possible factors: secondary contact between previously allopatric cytotypes (PETIT et al. 1999); and primary contact after the initial establishment of tetraploids (STUESSY et al. 2004). However, it is difficult to discriminate either of them or both together contributed to the contact zones of two cytotypes in this species before a corresponding genetic relationship was established.

Furthermore, our results also suggest that the contact zone between the two cytotypes of *A. przewalskianum* is devoid of triploid hybrids. Triploids have been found in mixed populations or in the contact zones of a few diploid-polyploid species complexes (HUSBAND and SCHEMSKE 1998). However, triploids are rarely found in the other autopolyploid species complexes (SOLTIS 1984; LUMARET et al. 1987; WOLF et al. 1990; van DIJK et al. 1992; NEFFA and FERNANDEZ 2001; STUESSY et al. 2004). The lack of the hybrid triploid may

be due to the low seed viability of triploids (van DIJK, HARTOG and van DELDEN 1992), asynchronous flowering, low mating success between different cytotype pollinations (HUSBAND and SCHEMSKE 1998) and other types of ecological isolation (BAACK 2005). Unfortunately, none of this information is available for *A. przewalskianum*. In addition to triploid hybrids generated between diploids and tetraploids on the 2x level, it is possible that crosses can also occur between 2x and 4x cytotypes on the 4x level via unreduced gametes in the diploid (RAMSEY and SCHEMSKE 1998). However, this sort of hybridization is cytologically difficult to detect. Overall, these interesting issues can be addressed and examined in the near future by using molecular approaches based on the current findings of diploid-polyploid distributions.

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