

CHROMOSOME NUMBERS AND KARYOTYPES OF *ALLIUM PRZEWALSKIANUM* POPULATIONS

CHENGQI AO^{1,2*}

¹Key Laboratory of Arid and Grassland Ecology of the Ministry of Education,
Lanzhou University, Lanzhou 730000, China

²School of Life and Environmental Sciences, Wenzhou University,
Zhejiang 325027, China

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In this study, somatic chromosome counts were determined in 302 individuals from 43 *Allium przewalskianum* populations; 90 were diploids ($2n = 16, 16 + 1B, 16 + 2B$) and 212 were tetraploids ($2n = 32$). Of the 43 populations, five were selected for karyotype analysis. Among them, the diploid plants have two karyotypes: $2n = 2x = 14m + 2st$ (2SAT) and $2n = 2x = 8m + 6sm + 2st$ (2SAT). The tetraploid has one: $2n = 4x = 28m + 4st$. Mixoploidy, Robertsonian translocations, and B chromosomes were reported for the first time. In combination with previous chromosome data, the present study reveals a uniform basic chromosomal number ($x = 8$) and uniformity of karyotypes (Stebbins's 2A type), indicating that speciation through polyploidization is less likely in *A. przewalskianum*, despite the highly diversified habitats it occupies.

Key words: *Allium przewalskianum*, chromosome number, karyotype, B-chromosome, Robertsonian translocation.

INTRODUCTION

Allium przewalskianum (sect. *Rhiziridium* G. Don, genus *Allium*, Alliaceae) is a perennial herb widely distributed in the Qinghai-Tibet Plateau and adjacent areas, including western China (Yunnan, Tibet, Sichuan, Shanxi, Ningxia, Gansu, Qinghai and Xinjiang), India and Nepal, at 2000–4800 m a.s.l. (Xu, 1980; Xue et al., 2000). The habitat it occupies is highly diversified, including dry slope, rock fissures, sandy soil and meadow. The plant propagates mainly through vigorous vegetative columniform bulbs. Within this species, three chromosome numbers (16, 32, 64) and one karyotype (Stebbins's 2A type) are known (Yang et al., 1998; Xue et al., 2000).

In the present study, somatic chromosome numbers were determined in 302 individuals from 43 populations of *A. przewalskianum* from the Qinghai-Tibet Plateau, and five populations were selected for karyotype analysis. Based on the available chromosome data (Yang et al., 1998; Xue et al., 2000 and the present study), the mechanisms of karyotype differentiation and population diversification were elucidated.

MATERIALS AND METHODS

The populations, along with their localities and habitats, are listed in Table 1. Voucher specimens are deposited in the Herbarium of the Northwest Plateau Institute of Biology, Chinese Academy of Science (HNWP). Root tips taken directly from living plants were pretreated in a of 1:1 (v/v) mixture of 8-hydroxyquinoline (0.002% w/v) and colchicine (0.05% w/v) for 2 h, fixed in 3:1 ethanol/acetic acid, and stored in 70% ethanol. After maceration in 1 mol/L HCl for 5 min at 60°C, the materials were stained and squashed with Carbol Fuchsin. For each plant the chromosomes of at least 5 cells were counted and measurements of at least 3 cells were made. Nomenclature for the centromeric positions of chromosome follows Levan et al. (1964) and the karyotype classification follows Stebbins (1971).

RESULTS

The numbers of chromosomes and numbers of individuals examined are listed in Table 1. In total, 302

*e-mail: aocq@wzu.edu.cn, aocq@tom.com

TABLE 1. Origins, habitats, numbers of chromosomes (2n) and numbers of individuals (n) examined in *A. przewalskianum*

| Population number | No. of chromosomes | Locality | Latitude, longitude, habitat and elevation |
|-------------------|--------------------|--|--|
| AO-03 | 32(3) | Ebo, Qilian Mt., Qinghai | N37°59.88'E100°45.92' Among grass, 3223 m |
| AO-14 | 16(1) 32(4) | Haiyan, Qinghai | N36°51.37'E101°03.25' Among grass, 2950 m |
| AO-23 | 32(1) | Tongren, Qinghai | N35°42.66'E102°17.65' Dry slopes in valley, 3020 m |
| AO-25 | 32(3) | Entrance of Maixiu forestland, Qinghai | N35°22.34'E101°57.75' Dry slopes, 2740m |
| AO-30 | 16(3) | Suburb of Henan, Qinghai | N34°44.20'E101°38.09' Gravelly slopes, 3600 m |
| AO-41 | 32(7) | Zeku, Qinghai | N35°16.50'E101°06.67' Among grass, 3500 m |
| AO-43 | 32(5) | Between Zeku and Maqin, Qinghai | N35°07.75'E100°43.66' Among grass, 3450 m |
| AO-52 | 32(6) | Maqin, Qinghai | N34°34.84'E100°33.33' Dry slopes in valley, 3430 m |
| AO-53 | 16(6) | Near Ning-Guo highway, Qinghai | N34°43.29'E100°48.92' Under cypress, 3500 m |
| AO-54 | 16(2) | Maixiu forestland, Qinghai | N35°15.78'E101°52.99' Under cypress in valley, 3170 m |
| AO-56 | 32(6) | Wutun, Tongren, Qinghai | N35°35.60'E102°03.16' Gravelly slopes, 2360 m |
| AO-62 | 32(8) | Lijie, Tongren, Qinghai | N35°26.77'E102°27.40' Gravelly slopes, 3080 m |
| AO-66 | 16(11) | Lintao, Gansu | N35°23.33'E 103°50.40' Grassy valley, 1880 m |
| AO-67 | 32(11) | Yuzhong, Gansu | N 35°46.80'E 104°01.38' Sunny slopes, 2450 m |
| AO-69 | 16(5) | Machang, Ledu, Qinghai | N36°25.01'E102°46.16' Dry slopes, 2560m |
| AO-70 | 16(4) | Sanhe, Pingan, Qinghai | N36°28.72'E102°02.34' Soil slopes, 2200 m |
| AO-72 | 16(8) 32(1) | Xining suburb, Qinghai | N36°33.79'E101°54.36' Grassy slopes, 2210 m |
| AO-75 | 32(5) | Huangzhong, Qinghai | N36°30.43'E101°37.90' On ridge of field, 2540 m |
| AO-77 | 16(5) 16+1B(1) | Longyang Valley, Qinghai | N36°12.80'E100°59.77' Dry slopes, 2850 m |
| AO-78 | 16(2) 32(13) | Qinghai Lake | N36°33.16'E100°43.76' Sandy soil, 3210 m |
| AO-80 | 32(8) | Gonghe, Qinghai | N36°22.38'E100°53.67' Fissures of rocks, 3490 m |

TABLE 1. (cont.)

| Population number | No. of chromosomes | Locality | Latitude, longitude, habitat and elevation |
|-------------------|---------------------------|------------------------------|---|
| AO-82 | 32(7) | Gamayangqu, Xinghai, Qinghai | N35°44.43'E100°13.40' Among grass, 3220 m |
| AO-85 | 32(7) | Daheba, Xinghai, Qinghai | N35°53.21'E099°40.59' Fissures of rocks, 3760 m |
| AO-89 | 32(7) | Chengduo, Qinghai | N33°20.52'E097°10.60' On rocky slopes, 4020 m |
| AO-92 | 32(9) | Xiewu, Yushu, Qinghai | N33°11.01'E097°23.64' Fissures of rocks, 4010 m |
| AO-95 | 32(8) | Suburb of Yushu, Qinghai | N33°00.20'E097°09.00' Fissures of rocks, 3610 m |
| AO-101 | 32(7) | Nangqian, Qinghai | N32°20.52'E096°25.85' Gravelly slopes, 3680 m |
| AO-103 | 16(11), 16+1B(2) 32(2) | Nangqian to Leiwuqi, Qinghai | N31°51.33'E096°17.74' Gravelly slopes, 3660 m |
| AO-106 | 32(8) | Batang, Yushu, Qinghai | N32°51.78'E096°57.46' Fissures of rocks, 3950m |
| AO-108 | 16(13) 16+1B(2) | Shiqu, Sichuan | N32°59.86'E098°20.37' Fissures of rocks, 3970 m |
| AO-117 | 16+1B(12) 16+2B(2) | Jiangda to Changdu, Tibet | N31°26.61'E098°09.07' Gravelly slopes, 3640 m |
| AO-119 | 32(23) | Changdu, Tibet | N31°08.85'E097°01.17' Fissures of rocks, 3630m |
| AO-121 | 32(8) | Leiwuqi, Tibet | N31°12.32'E096°36.06' Fissures of rocks, 3800m |
| AO-128 | 32(3) | Dingqing, Tibet | N31°18.36'E095°45.66' Grassy slopes, 3680 m |
| AO-130 | 32(13) | Dingqing to Baqing, Tibet | N 31°41.34'E095°01.49' Fissures of rocks, 3860 m |
| AO-132 | 32(14) | Suoxian, Tibet | N31°48.80'E093°44.46' Fissures of rocks, 3970 m |
| AO-134 | 32(3) | Naqu, Tibet | N 31°45.29'E092°46.55' Gravelly slopes, 4220 m |
| AO-142 | 32(4) | Jiangzi to Langkazi, Tibet | N28°50.99'E089°55.04' Fissures of rocks, 4300 m |
| AO-157 | 32(3) | Dangxiong, Tibet | N30°32.36'E091°07.67' Gravelly slopes, 4310 m |
| AO-158 | 32(1) | Xiangride, Dulan, Qinghai | N36°00.82'E097°53.44' Fissures of rocks, 3050 m |
| AO-159 | 32(2) | Chahanwusu, Dulan, Qinghai | N36°14.68'E098°06.37' Fissures of rocks, 3250 m |
| AO-160 | 32(1) | Delingha, Qinghai | N37°26.83'E97°20.28' Fissures of rocks, 3310 m |
| AO-161 | 32(1) | Wulan, Qinghai | N37°04.31'E098°52.46' Fissures of rocks, 3470 m |

individuals from 43 populations were examined, of which 90 were diploids ($2n = 16, 16 + 1B, 16 + 2B$) and 212 tetraploids ($2n = 32$) based on $x = 8$.

AO-14, AO-72, AO-78 and AO-103 are mixoploid populations with diploids and tetraploids, reported in the species for the first time here.

TABLE 2. Ploidy level, chromosomal numbers and karyotype for 16 populations of *A. przewalskianum*

| Population | Ploidy and karyotype formula | Karyotype classification | Literature cited |
|------------------------------------|--|--------------------------|--------------------|
| Qinghai, Yushu (AO-95) | $2n=4x=32=28m+4st$ | 2A | The present study |
| Qinghai Lake (AO-78) | $2n=2x=16=8m+6sm+2st$ (2SAT) $2n=4x=32=28m+4st$ | 2A 2A | The present study |
| Qinghai, Nangqian-Leiwuqi (AO-103) | $2n=2x=6=14m+2st$ (2SAT) $2n=2x=16=14m+2st$ (2SAT)+1B $2n=4x=32=28m+4st$ | 2A 2A 2A | The present study |
| Qinghai, Huangyuan | $2n = 2x = 16 = 14m + 2st$ (2SAT) | 2A | Xue et al. (2000) |
| Qinghai, Xining | $2n=2x=6=14m+2st$ (2SAT) | 2A | Xue et al. (2000) |
| Qinghai, Gonghe | $2n=2x=16=12m+2sm+2st$ (2SAT) | 2A | Xue et al. (2000) |
| Qinghai, Maqin | $2n=4x=2x=24m+4sm+4st$ | 2A | Xue et al. (2000) |
| Qinghai, Yushu -1 | $2n=4x=32=28m+4st$ (2SAT) | 2A | Xue et al. (2000) |
| Qinghai, Yushu -2 | $2n=4x=32=26m+2sm+4st$ | 2A | Xue et al. (2000) |
| Qinghai, Yushu -3 | $2n = 4x = 32 = 24m + 4sm + 4st$ | 2A | Xue et al. (2000) |
| Qinghai, Nangqian -1 | $2n = 4x = 32 = 28m + 4st$ (2SAT) | 2A | Xue et al. (2000) |
| Qinghai, Nangqian -2 | $2n=8x=64=54m+2sm+8st$ | 2A | Xue et al. (2000) |
| Tibet, Jiangda (AO -117) | $2n=2x=16=14m+2st+1B$ $2n=2x=16=14m+2st+2B$ | 2A 2A | The present study |
| Tibet, Zhagyab | $2n=4x=32=28m+4st$ (2SAT) | 2A | Yang et al. (1998) |
| Sichuan, Shiqu (AO -108) | $2n=2x=16=14m+2st$ (2SAT) $2n = 2x = 16 = 14m + 2st$ (2SAT)+1B | 2A 2A | The present study |
| Sichuan, Lixian | $2n=4x=32=28m+2sm+2st$ (2SAT) | 2A | Yang et al. (1998) |

Populations AO-78 (Qinghai Lake), AO-95 (Yushu), AO-103 (Nangqian-Leiwuqi), AO-108 (Shiqu) and AO-117 (Jiangda) were chosen for karyotype analysis. In analyzing the chromosome constitution of the five selected populations, we found that the arm ratios of only one pair of chromosomes in diploids, and two pairs in tetraploids, exceeded 2.0, indicating very high intrachromosomal symmetry in *A. przewalskianum*. However, the ratio of longest to shortest chromosome is < 2 (Figs. 10-16). Both the above-mentioned characteristics determine a karyotype classification of 2A for all diploid or tetraploid plants (Stebbins, 1971) (Tab. 2).

The karyotypes of the diploid plants belong to two types. One is formulated $2n = 2x = 14m + 2st$ (2SAT). Population AO-108 (Shiqu), population AO-117 (Jiangda) and population AO-103 (Nangqian-Leiwuqi) exhibit this type, though in population AO-117 a pair of subterminal chromosomes is not satellited (Figs. 11, 12). Satellites are sometimes indistinct due to the effect of time and temperature of pretreatment in the field. The presence of satellites was highly variable, and their presence or absence cannot be used to characterize species or taxonomic groups (Fritsch and Astanova, 1998). The other type is formulated $2n = 2x = 8m + 6sm + 2st$ (2SAT), which means that 6 metacentric chromosomes of the first karyotype are replaced by 6 submetacentrics.

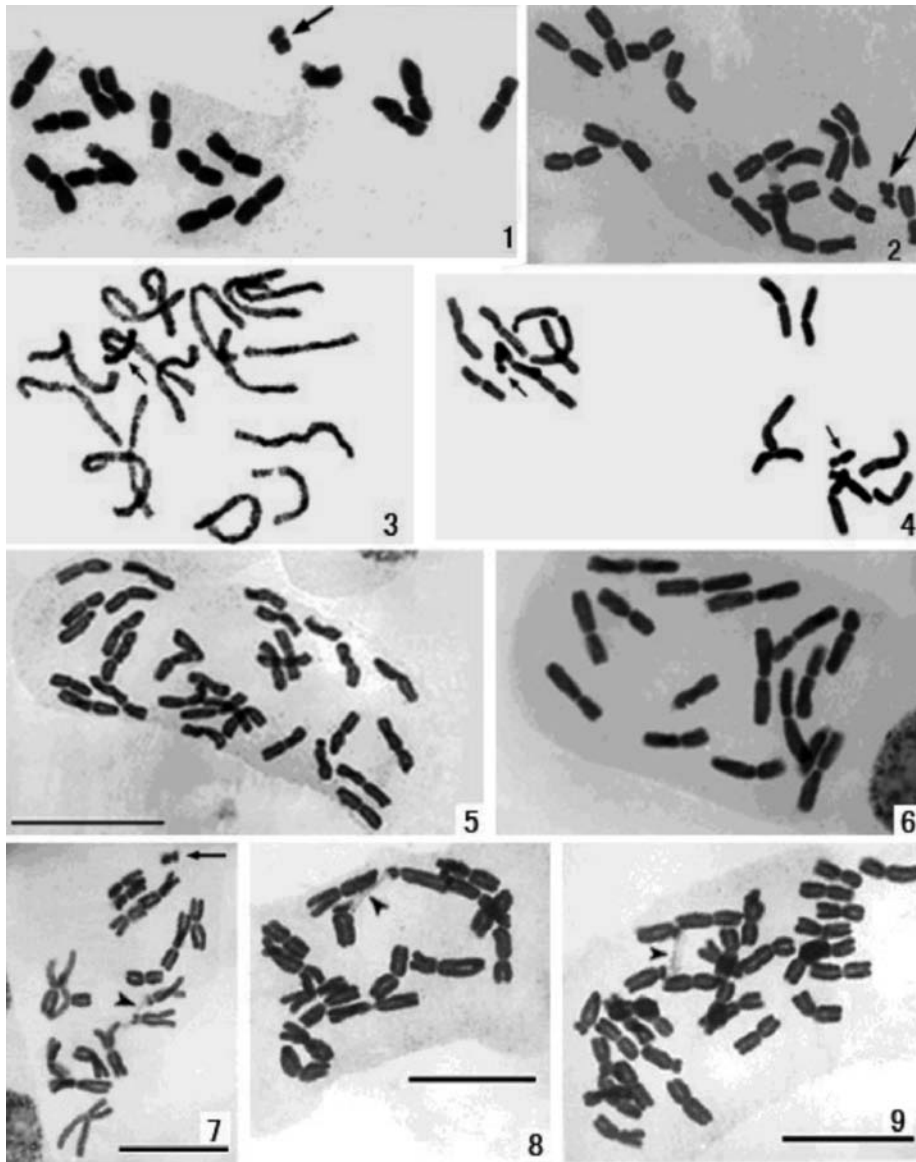
Only population AO-78 is included in the second type. The tetraploid plants of all populations possess a uniform karyotype: $2n = 4x = 28m + 4st$.

In population AO-108, one of the plants had an additional chromosome in its complement; it appears to be shorter than the others, obviously metacentric (Figs. 1, 10). The supernumerary chromosome in the diploid most likely represented a B chromosome. So did a number of diploids in population AO-117 and population AO-103, but the B chromosomes were submetacentric (Figs. 2, 4, 7, 11, 12).

The karyomorphological images of population AO-103 (Figs. 7-9) suggest a simple Robertsonian relationship, since the two telocentric chromosomes were probably derived from the splitting of one metacentric.

DISCUSSION

Of the 302 individuals surveyed, 212 are tetraploids. Clearly polyploidy prevails in *A. przewalskianum*. In the four mixed populations with diploids and tetraploids, none of the tetraploid plants is distinguishable morphologically from the diploid of the same population, suggesting that the tetraploids may be individuals spontaneously occurring through autopolyploidy.

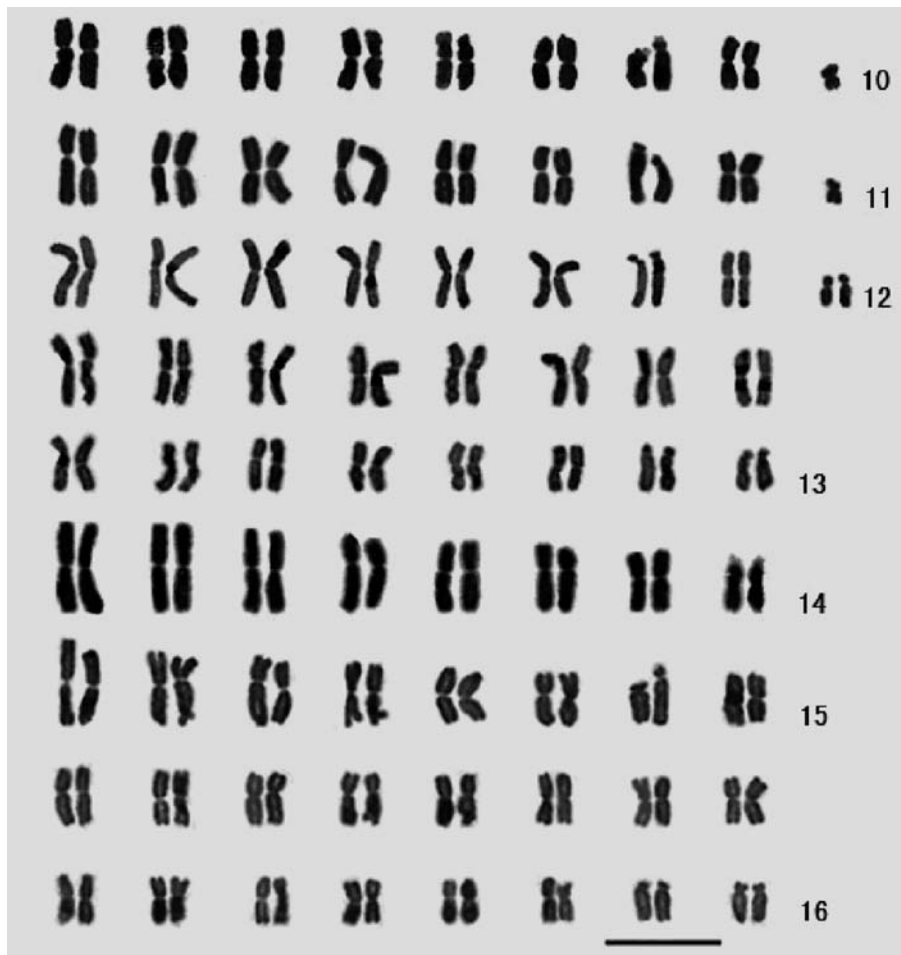


Figs. 1–9. Chromosomes of *A. przewalskianum*. **Fig. 1.** Population AO-108 (Shiqu). **Figs. 2–4.** Population AO-117 (Jiangda). **Fig. 3.** Prophase chromosomes; other figures are metaphase chromosomes. Arrows – B chromosomes; arrowheads – Robertsonian translocation. **Fig. 5.** Population AO-95 (Yushu). **Fig. 6.** Diploid of population AO-78 (Qinghai Lake). **Figs. 7–9.** Population AO-103 (Nangqian-Leiwuqi). Bars = 10 μ m. Fig. 5 bar applies to Figs. 1–6.

In general, a triploid arises through hybridization of a diploid and a tetraploid (Stebbins, 1971, 1974; Grant, 1981). Therefore most triploid plants occur in mixoploid populations that contain diploids and tetraploids (Grant, 1981; Hong, 1990). Triploid plants can also be produced via an unreduced egg united with normal reduced pollen (Grant, 1981). This situation occurs in populations involving a single diploid, and is less frequent in natural plant populations (Liu, 2004; Grant, 1981; Hong, 1990), but has been recorded in the *Aster ageratoides* complex from Taiwan (Soejima and

Peng, 1998) and in *Ligularia* (Liu, 2004). However, no triploid plant was encountered in either mixoploid or diploid populations of *A. przewalskianum* after a great many individuals were karyomorphologically examined. Hence it can be inferred that hybridization has played a minor role in the population diversification of *A. przewalskianum*.

All available karyotype data on *A. przewalskianum* are listed in Table 2, revealing a uniform 2A type. *A. przewalskianum* has a more symmetrical karyotype in *Allium* sect. *Rhiziridium*, compared with those possessing a 2B karyotype, such as



Figs 10–16. Karyotypes of *A. przewalskianum*. **Fig. 10.** Population AO–108 (Shiqu) [$2n = 2x = 14m + 2st (2SAT) + 1B$]. **Fig. 11.** Population AO–117 (Jiangda) ($2n = 2x = 14m + 2st + 1B$). **Fig. 12.** Population AO–117 (Jiangda) ($2n = 2x = 14m + 2st + 2B$). **Fig. 13.** Population AO–95 (Yushu) ($2n = 4x = 28m + 4st$). **Fig. 14.** Diploid of population AO–78 (Qinghai Lake) [$2n = 2x = 10m + 4sm + 2st (2SAT)$]. **Fig. 15.** Diploid of population AO–103 (Nangqian-Leiwuqi) [$2n = 2x = 14m + 2st (2SAT)$]. **Fig. 16.** Tetraploid of population AO–103 (Nangqian-Leiwuqi) ($2n = 4x = 28m + 4st$). Bar = 10 μ m.

A. strictum Schrader (Tolgor et al., 1994). Therefore it may be considered to be less highly evolved in the section, from the standpoint of Stebbins (1971). However, its polyploid status may help it stabilize and establish in new habitats, extending its area of distribution.

Although naturally occurring aneuploids are exceedingly rare in plants and have not been documented in *A. przewalskianum* (Yang et al., 1998; Xue et al., 2000), Robertsonian translocations were clearly displayed in population AO–103, where one metacentric splits into two telocentrics or vice versa. Robertsonian translocations may result in aneuploid reduction of chromosomal number, so it can be inferred that aneuploids ($2n = 15$, $2n = 31$) will probably appear in *A. przewalskianum*, provided a large number of individuals are examined.

There are many records of B chromosomes in the genus as a whole (Jones and Rees, 1982), but their presence is not common in sect. *Rhiziridium*. Among the members of this section, a single B was seen in somatic cells of *A. barsczewskii* (Sarker et al., 1997).

In this work, 1 or 2 B chromosomes were detected in mitotic cells of *A. przewalskianum* for the first time. B chromosomes are dispensable supernumerary chromosomes which do not recombine with A chromosomes and which follow their own evolutionary pathway. In plants, principally from rye and maize, the recently emerging view is that B chromosomes are parasitic elements and that there is a host-parasite relationship between the A and B chromosomes (Jones and Houben, 2003). B chromosomes have been reported in over a thou-

sand plant species (Jones and Rees, 1982), but it remains unclear why they are so prevalent, what their mode of transmission is, and their effect on male fertility.

Sixteen populations investigated here or previously show a high degree of karyotypic differentiation (Tab. 2). Habitat diversification, vegetative propagation and polyploidization can well explain the mechanisms of karyotypic differentiation. *A. przewalskianum* is widely distributed in the Qinghai-Tibet Plateau, and the variety of environmental conditions inevitably causes habitat diversification, conducive to the formation of chromosomal structural aberrations (Tzanoudakis, 1983). Moreover, reproduction through vegetative bulbs as compensation for reduced fertility brings about and stabilizes chromosomal aberrations (Stebbins, 1950; Zhu and Xu, 1999). Because multiple alleles at each locus mask deleterious mutations, polyploids can tolerate more chromosomal structural aberrance than their diploid progenitors can. Thus, chromosomal structural aberrations in polyploids are more frequent than in diploids (Hong, 1990). Differential banding and in situ hybridization studies are needed to elucidate the cause of karyotypic diversity within this species.

New plant species are produced either through autopolyploidy or through hybridization of two existing species (Stebbins, 1974). However, the uniform basic chromosomal number ($x = 8$) and uniformity of karyotypes (Stebbins' 2A type) in all the investigated populations indicate that speciation through polyploidization is less possible in *A. przewalskianum*, despite the highly diversified habitats it occupies and apparent karyotype differentiation.

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