广 西 植 物 Guihaia 24(5):422－425 2004 年 9 月

丰都车前的细胞学研究,兼论它的多倍体起源

王恒昌,孟爱平,李建强*,王 勇,陶 勇

(中国科学院武汉植物园,湖北武汉 430074)

摘 要:对长江三峡库区特有濒危植物丰都车前(*Plantago erosa* var. *fengdouensis*)进行了细胞学研究,包括有丝分裂和减数分裂两方面。丰都车前可能为一异源四倍体。其核型公式为 2n=4x=24=12m(2sat)+12sm(2sat),属于 2A 型。极端的环境压力尤其是季节性的水淹可能是此四倍体物种形成的主要原因。对同属内的另一物种车前(*P. asiatica*)进行了比较观察,确证车前为六倍体。该实验为丰都车前的保育遗传学研究提供了细胞学证据。

关键词:丰都车前;三峡库区;多倍体起源

中图分类号:Q943 **文献标识码**:A **文章编号**:1000-3142(2004)05-0422-04

Cytological studies of *Plantago erosa* var. *fengdouensis*, with special reference to its polyploid origin

WANG Heng-chang, MENG Ai-ping, LI Jian-qiang*,
WANG Yong, TAO Yong

(*Wuhan Botanical Garden, Chinese Academy of Sciences*, Wuhan 430074, China)

Abstract: Cytological studies including mitosis and meiosis of *Plantago erosa* var. *fengdouensis*, an endangered plant native to the Three-Gorge Reservoir Area of China, have been investigated. It may be an allotetraploid. The karyotype can be formulated as 2n=4x=24=12m(2sat)+12sm(2sat), belonging to 2A type. Extreme environmental impetus especially the seasonal flooding may play a principal role in the polyploid formation of it. Chromosome number of *P. asiatica* is reconfirmed, and *P. asiatica* is a hexaploid. This study will supply a cytological evidence for the further genetics conservation research of *Plantago erosa* var. *fengdouensis*.

Key words: *Plantago erosa* var. *fengdouensis*; Three-Gorge Reservoir Area; polyploid origin

1 Introduction

Plantago L. (Plantaginaceae) is a cosmopolite genus. There are more than 190 species in total genus, and 20 occur in China (Hu *et al.*, 2002). According to the classification standard of Zheng *et al.* (1992), *P. erosa* Wall. ex Roxb. var. *fengdouensis* Z. E. Zhao et Y. Wang was described. The new variety is characterized by lanceolate, subincised and trinerved leaves, flowers without pedicel, the styles of flowers being 4～7 mm long and gla-

Received date: 2003-09-24 **Accepted date**: 2003-12-24

Foundation item: Wuhan Botanical Garden director fund(01035108,01015110); Chinese Academy of Sciences(KSCX2-SW-104).

First auther: Wang Heng-chang(1967-), Male, born in Xiantao city, Hubei Province, Doctoral student, Associate professor, working in plant systematics and taxonomy. * Author for correspondence E-mail: jianqiangl@hotmail.com

brous, and flooding tolerant (Zhao *et al.*, 2002). Only two populations of the variety were discovered in two small islands of two counties along the Yangtze River. One is located in Fengdou of Chongqing city, another in Zhongxian. There are over 100 km between the two localities, which are located in a submergible plain of the Three-Gorge Reservoir Area of China. In December, 2002, before the famous Dam began to retain water, one of authors of this paper transplanted most of wild individuals of the variety into Wuhan Botanical Garden, Chinese Academy of Sciences (CAS), and cultivated them under a semi-natural condition. In Plantago, the basic chromosome number is x=6 for most species and polyploids are common from 2n=4x=24 to 2n=16x=96 (Fedorov, 1974). *P. erosa* is a tetraploid with 2n=4x=24 (Hong and Zhang, 1990). This study aims at through a cytological research on the variety, (1) providing additional evidence for the further research on genetic conservation and taxonomical re-evaluation of this endemic, rare and endangered taxon of the Three-Gorge Reservoir Area; (2) presenting an example for probing into the origin and evolution of tetraploid group that confined to some constricted ecological habitat. For the later purpose, another species *P. asiatica* L. is also investigated.

2 Materials and Methods

The plants investigated were cultivated in Wuhan Botanical Garden, CAS. Young growing inflorescences were obtained for cytological observation and the voucher specimens were deposited in HIB.

For somatic chromosome observation, the materials were pretreated in a saturated solution of p-Dichlorobenzene for 2.5 h before fixed in Carnoy I (glacial acetic acid : absolute ethanol = 1 : 3) at 20 ℃ for 1 h. For meiotic process investigation, the young anthers were fixed in Carnoy I for 0.5 h. Then we used wall degradation and hypotonic treatment to prepare chromosome samples. More than 10 metaphase cells were detected to determine the chromosome number. The symbols classification standard used to describe the karyotypes followed Stebbins (1971) and Levan *et al.* (1964), the karyotype asymmetry index followed Arano (1963).

3 Results

3.1 Mitosis

The chromosome number of *P. erosa* var. *fengdouensis* investigated in the metaphase was counted to be 2n=4x=24 (Plate Ⅰ:3). It would be a tetraploid. The whole 24 chromosomes could be roughly arranged into 6 groups of 4 homologues but there existed obvious heteromorphy between the two pairs of homologues in the third and sixth groups (Plate Ⅰ: 10). According Tanaka (1971, 1977), the resting nuclei and prophase chromosomes of *P. erosa* var. *fengdouensis* were categorized to be complex chromocenter type and interstitial type respectively (Plate Ⅰ: 1, 2), the karyotype can be formulated as 2n=4x=24=12m (2sat)+12sm (2sat). The chromosome morphology and karyotype are shown (Plate Ⅱ: 10). The karyograms are listed in Table 1. The ratio of the longest chromosome to the shortest (L/S) is 1.44, and the percentage of Arm ratio over 2 : 1 is 0.375, so the karyotype belongs to 2A type. The index of karyotype asymmetry is 63.41%.

3.2 Meiosis

The chromosome number of *P. erosa* var. *fengdouensis* investigated at metaphases is consistently counted to be 2n=4x=24 (Plate Ⅰ: 5, 6). At the prophase of meiosis, the chromosomes are diffuse with chromatin slender and winded each other (Plate Ⅰ: 4). At metaphase I, the configuration of *P. erosa* var. *fengdouensis* is almost uniform (Plate Ⅰ: 5, 6). No tetravalent but bivalents occur, and the bivalents are sticklike. It seems that *P. erosa* var. *fengdouensis* is more an allotetraploid than an autoploid or a segmental allotetraploid. We also investigate the stages of anaphase I and anaphase Ⅱ of *P. erosa* var. *fengdouensis*, the division proces-

ses are found to be normal (Plate Ⅰ: 7, 9), while very seldom lagging chromosomes are observed (Plate Ⅰ: 8). Meanwhile, we make a statistic of the pollen grain fertility and find they are almost normal also.

Table 1 The karyomorphological parameters of *P. erosa* var. *fengdouensis*

Group No.	Relative length %	Arm ratio	Classification
1	19.45	1.47	m
2	17.51	2.04	sm
3*	8.14	1.35	m
	8.05	2.14	sm
4	15.59	1.32	m
5	15.88	2.08	sm
6	7.81	1.27	m
	7.57	2.11	sm

The value is based on the means of measurements of the best five cells. * chromosome with satellite

Additionally, we observe the meiosis of *P. erosa* var. *fengdouensis* and confirm the bivalent counts of it are 18. The species would be a hexaploid with 2n=6x=36 (Plate Ⅱ: 11, 12, 13, 14).

4 Discussion and Conclusion

Confined to a labile biota, *P. erosa* var. *fengdouensis* is not an aggressive taxon, and may at most be a kind of moderate success of tetraploid. The two seasonal islands (app. 1 500 × 150 m^2) where the variety is located remain to be land most time of a year while submerge when flooding occurs from June to late August. The aerial portions of the plants of the variety die back and disarticulate from the rootstocks at autumn with seedlings that keep evergreen from winter to spring. The growth habits of *P. erosa* var. *fengdouensis* hence present an adaptive mechanism of counter-seasonal growing. Transplanted in Wuhan Botanical Garden, it doesn't spread beyond the place it is grown. The average of seed germination is about 20% and greatly lower than that of *P. asiatica* (app. 95%). Except *P. asiatica*, a vigorous and fertile weed everywhere, no other *Plantago* species were found companying with the varieties all over the wild region. The two *Plantago* groups have a vicariance distribution. It is interesting whether the two taxa have relation to each other in evolutionary origin. The chromosome numbers of *P. asiatica* reported by various authors seemed to be ambiguous (Chen, 1988, 1989, 2n=2x=12; Matsuo & Noguchi, 1989; Nishikawa, 1990, 2n=4x=24; Probatova, 1996, 2n=6x=36). Thereby a re-examination of its ploidy is needed.

Since *P. erosa* var. *fengdouensis* is proposed to be an allotetraploid, it may not derive from the diploid simple doubling of *P. asiatica* even though the later may possess diverse ploidy populations including diploid as Chen (1988, 1989) reported. Obviously different in morphological characteristic and ecological habit, genetic introgression in tetraploid level between the two taxa seems to be impossible. Moreover, we examine *P. asiatica* is a hexaploid, so this assumed possibility can basically be excluded. Briefly, the ancestral diploid progenitors of *P. erosa* var. *fengdouensis* would have been extinct. Interestingly, there are two *P. erosa* var. *fengdouensis* populations distributed in two relatively isolated islands along the Yangtze River, where the ecological factors are found to be very similar. This seems to infer that environmental impetus especially the extreme flooding has played an important role in the origin of the tetraploid *P. erosa* var. *fengdouensis*.

The taxonomical position of *P. erosa* var. *fengdouensis* should be recognized, though whether it is a variety or a subspecies needs a further critical circumscription. From the cytogenetic point of view analysed above, the taxon possesses some relatively independent cytological, as well as distinct morphological and ecological features. Nonetheless, as Stebbins (1979) pointed out, these kinds of populations need to be studied more carefully, particularly with respect to differences in genes coding for isozymes (or plus molecular marker) and ranges of ecological tolerance.

Acknowledgements We gratefully thank Prof. Z. E. Zhao for providing detailed field information.

References:

Arano H. 1963. Cytological studies in subfamily Carduoideae (Compositae)of Japan IX[J]. *Bot Mag(Tokyo)*, **76**: 32—39.

Chen RY. 1988. G-Band staining of chromosomes in some higher plants with special reference to Lilium davidii[J]. *Kromosomo*, **50**: 1 635—1 651.

Chen RY. 1989. A further research of plant chromosome G-banding. In: Hong DY(ed). Plant Chromosome Research [M]. Beijing: Science Press, 187—193. (in Chinese).

Fedorov A. 1974. Chromosome numbers of flowering plants[M]. Koenigstein: Otto Koeltz Science Publishers, 418—419.

Hong DY. 1990. Plant cytotaxonomy[M]. Beijing: Science Press. (in Chinese).

Hong DY, Zhang SZ. 1990. Observations on chromosomes of some plants from Western Sichuan[J]. *Cathaya*, **2**: 191—197.

Hu CC, Tsui HP, Li ZY. 2002. Flora Reipublicae Popularis Sinicae[M]. Beijing: Science Press, **70**: 318—319. (in Chinese)

Levan A, Fredge K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes[J]. *Hereditas*, **52**: 201—220.

Matsuo K, Noguchi J. 1989. Karyotype analysis of several Plantago species in Japan, with special reference to the taxonomic status of *Plantago japonica*[J]. *Journal of Phytogeography and Taxonomy*, **37**: 27—35.

Nishikawa T. 1990. Chromosome counts of flowering plants of Hokkaido (13)[J]. *Journal of Hokkaido University of Education: Section IIB*, **40**: 19—30.

Probatova NS, Rudyka EG, Sokolovskaya AP. 1996. Chromosome numbers in synanthropic plants from the Russian Far East[J]. *Botaničeskij Žurnal(Moscow & Leningrad)*, **81**(5): 98—101.

Stebbins GL. 1971. Chromosomal evolution in Higher Plants [M]. London: AddisonWesley.

Stebbins GL. 1979. Polyploid in plant: unsolved problems and prospects[M]. In: Lewis WH(ed). Polyploidy. New York & London: Plenum press, 495—528.

Tanaka R. 1971. Types of resting nuclei in Orchidaceae[J]. *Bot Mag(Tokyo)*, **84**: 118—132.

Tanaka R. 1977. Recent karyotype studies. In: Ogawa K *et al*. (eds). Plant Cytology[M]. Tokyo: Asakura Shoten.

Zhao ZE, Wu JQ, Wang Y. 2002. New taxa of Lysimachi and Plantago in the Three-Gorge Reservoir Area[J]. *Jounal of Wuhan Botanical Research*, **20**: 263—264. (in Chinese, English abstr)

Zheng TK, Tanaka T, Kang TG. 1992. Studies on the Plantago Plants in China[J]. Shengyang: Liaoning Publishing House of Science and Technology. (in Chinese)

(上接第421页 Continue from page 421)

段，还有待于进一步将RAPD标记转换成更稳定、可靠的SCAR标记。

参考文献：

Da 党本元，胡赞民，周弈华，等. 1998. 王百合单染色体DNA文库构建[J]. 科学通报，**43**(3)：272—276.

Guo G(郭 歌)，Chen CB(陈成彬)，Li XL(李秀兰)，*et al*. 1998. Cloing of the telomere associated sequences of rye B-chromosomes(黑麦B染色体端粒相关序列的克隆)[J]. *Acta Botanica Sinica*(植物学报)，**40**(12)：1 123—1 128.

Li XY(李湘阳)，Zhou J(周 坚). 2002. Microdissection and PCR amplification of single *Cunninghamia lanceolata* chromosome(杉木单染色体的显微分离及体外扩增)[J]. *Forest Research*(林业科学研究)，**15**(6)：746—750.

Li XY(李湘阳)，Zhou J(周 坚)，Yuan HS(袁汉升)，*et al*. 2003. RAPD Studies on the amplified product of single microdissected *Cunninghamia lanceolata* chromasome(杉木单染色体扩增产物的RAPD分析)[J]. *Journal of Nanjing University*(*Natural Sciences Edition*)(南京林业大学学报)(自然科学版)，**27**(3)：55—58.

Ma YZ(马有志)，Xu QF(徐琼芳)，Xin ZY(辛志勇)，*et al*. 1999. Isolation of wheat chromosome by argon-ion laser beam(小麦染色体显微激光分离)[J]. *Acta Genetic Sinica*(遗传学报)，**26**(1)：43—48.

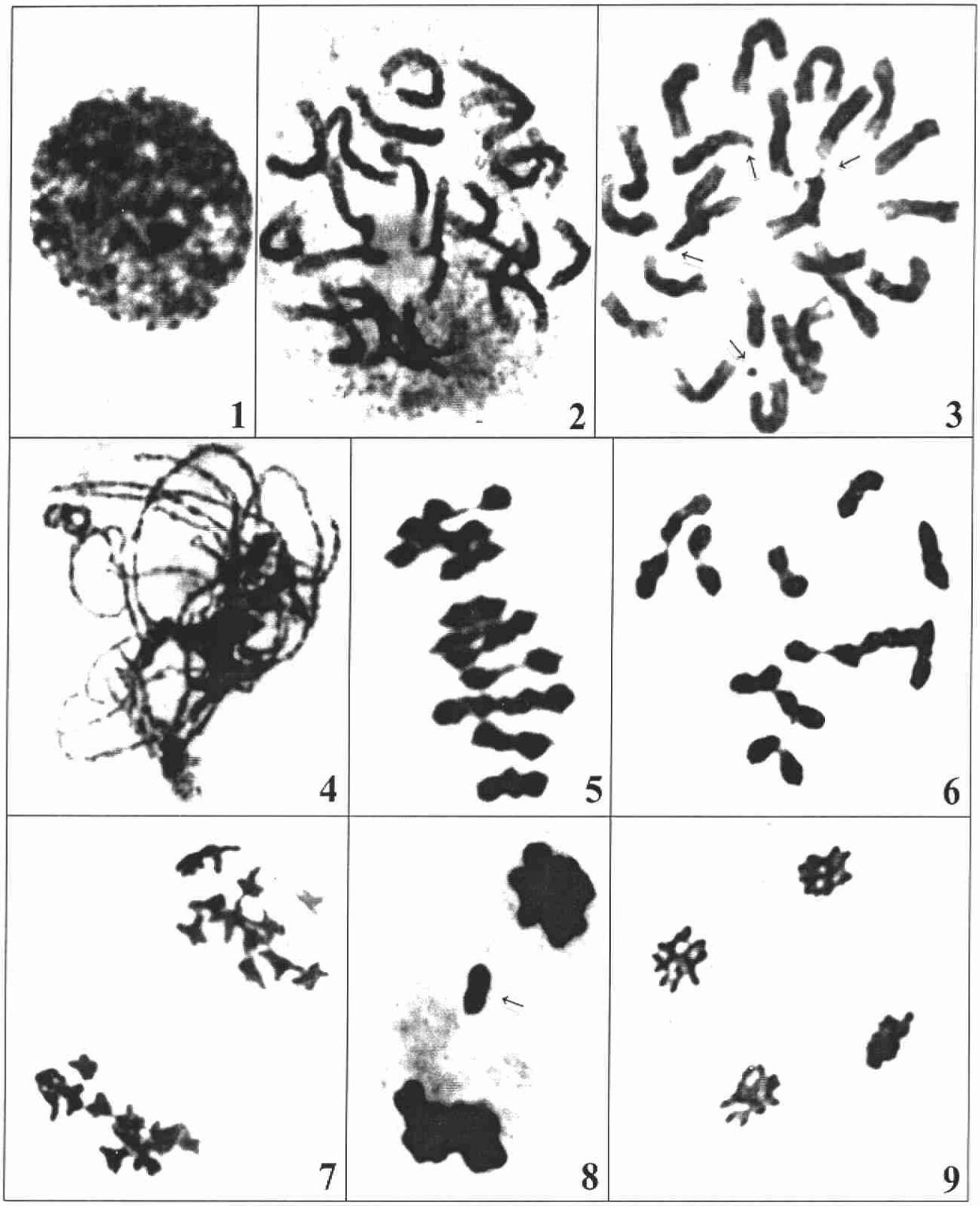
Sandery MJ, Forster JW, Macadam SR, *et al*. 1991. Isolation of a sequence common to Aand B-chromosomes of rye(*Secale cereale*) by microcloning[J]. *Plant Mol Biol Rep*, 9: 21—30.

Tian C(田 毅)，Liu GT(刘广田). 1997. The cloning of wheat HMW-GS1Dx5 gene fragement using chromosome microdissection(小麦染色体显微切割和微克隆方法的建立及HMW-GSID5亚基基因克隆)[J]. *Journal of Agricultural Biotechnology*(农业生物技术学报)，**5**(4)：307—312.

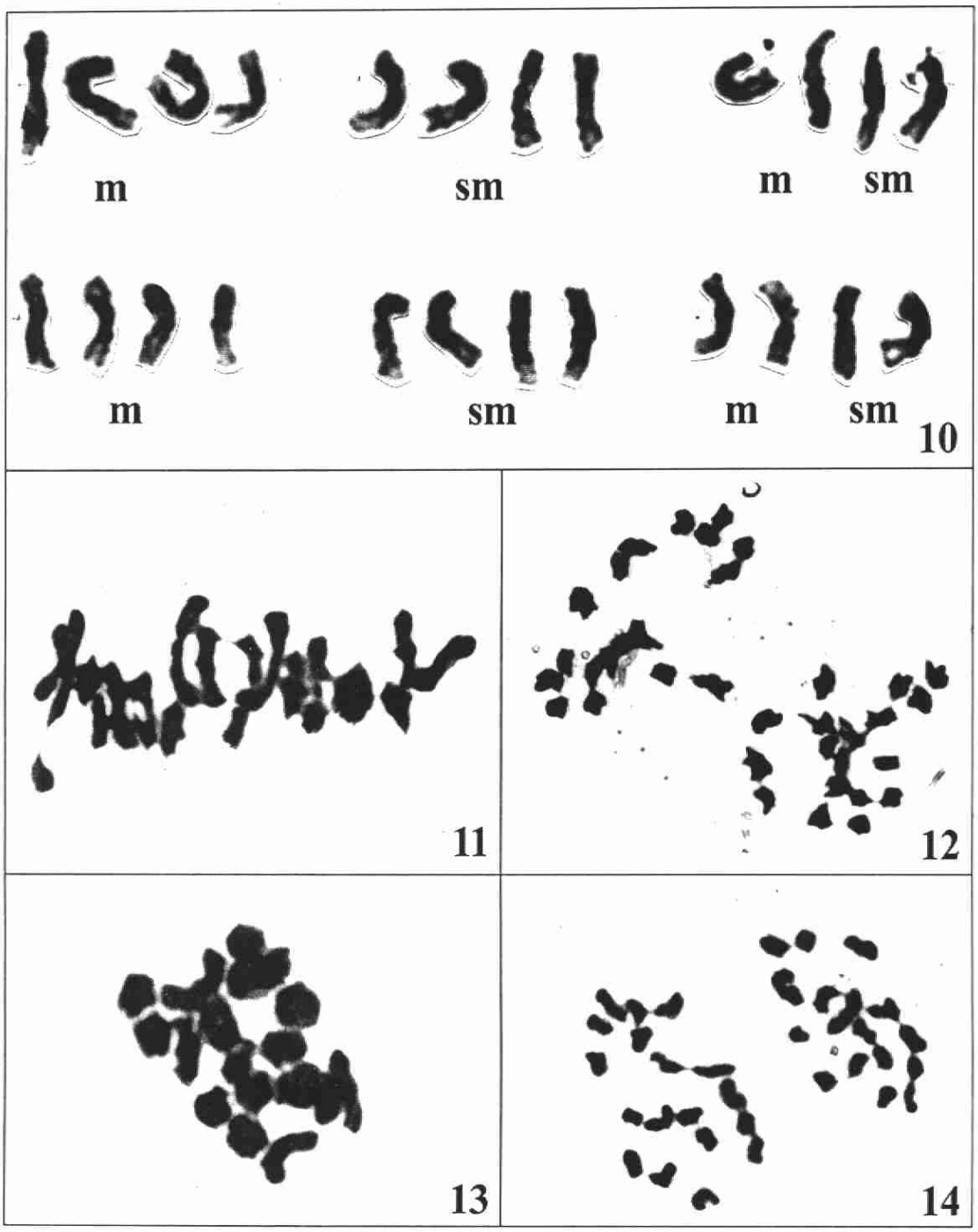
Telenius H, Carter NP, Bebb CE, *et al*. 1992. Degerate oligonucleotide primed PCR: general amplification of target DNA by a single degenrate primer[J]. *Genomic*, **13**: 718—725.

Williams JGK, Kubelik AR, Livak KJ, *et al*. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers[J]. *Nucleic Acida Res*, **18**: 6 531—6 535.

Welsh J, McCLelland M. 1991. Genomic fingerpriting with AP-PCR using pairwise combination of primers: Aplication to genetic mapping of the mouse[J]. *Nucleic Acids Res*, **19**: 5 275—5 279.



1～3. Mitosis of somatic chromosomes in *P. erosa* var. *fengdouensis*: 1. Resting nucleus; 2. Prophase; 3. Metaphase (Arrows show chromosomes with satellite), 2n=4x=24. 4～9. Meiosis stages: 4. Prophase; 5. Lateral view of Metaphase I (Showing 6 pairs of bivalents); 6. Apical view; 7, 8. Anaphase I (Arrow showing lagging chromosome); 9. Anaphase Ⅱ.



10. Karyogram of *P. erosa* var. *fengdouensis*. 11～14. Meiosis chromosome of *P. asiatica*. 11, 13. metaphase 1; 12, 14. Anaphase Ⅰ. (Photos magnification: for Figs. 1～10. ×2 000; for Figs. 11～14. ×1 810).