

NEW CHROMOSOME COUNTS IN FIVE SPECIES OF OXYTROPIS (FABACEAE) IN IRAN

M. Ranjbar, R. Karamian & S. Bayat

Received 20 12 2009. Accepted for publication 03 03 2010

Ranjbar, M., Karamian, R. & Bayat, S. 2010 06 30: New chromosome counts in five species of *Oxytropis* (Fabaceae) in Iran. *Iran. J. Bot.* 16 (1): 42-48. Tehran.

In this study original mitotic chromosome counts are presented for 4 *Oxytropis* species from 4 sections of the genus *Oxytropis* in Iran including: *O. bicornis* (*O. sect. Oxytropis*) ($2n = 2x = 16$), *O. suavis* (*O. sect. Ortholoma*) ($2n = 2x = 16$), *O. aucheri* (*O. sect. Eumorpha*) ($2n = 2x = 16$) and *O. karjaginii* (*O. sect. Janthina*) ($2n = 4x = 32$). This report is the first cytogenetic analysis of these taxa. All taxa (with exception of *O. karjaginii*) are diploid and possess $2n = 2x = 16$ chromosome number, consistent with the proposed base number of $x = 8$. In addition, meiotic chromosome count and behavior are presented for *O. masanderanensis* (*O. sect. Ortholoma*) ($2n = 2x = 16$). Although this taxon displayed regular bivalent pairing and chromosome segregation at meiosis, but some abnormalities were observed.

Massoud Ranjbar (correspondence, ranjbar@basu.ac.ir) and Roya Karamian & Soheila Bayat, Department of Biology, Herbarium division, Bu-Ali-Sina University, P.O. Box 65175/4161, Hamedan, Iran.

Key words. meiotic behavior, mitosis, morphology, *Oxytropis*, Iran.

گزارش شمارش کروموزومی جدید برای ۵ گونه از جنس **Oxytropis** در ایران

دکتر مسعود رنجبر، استادیار گروه زیست شناسی دانشگاه بوعلی سینا.

دکتر رویا کرمان، دانشیار گروه زیست شناسی دانشگاه بوعلی سینا.

سهیلا بیات، دانشجوی گروه زیست شناسی دانشگاه بوعلی سینا.

در این مطالعه، گزارش عدد کروموزومی میتوز برای ۵ گونه متعلق به ۴ بخش از جنس *Oxytropis* در ایران شامل *O. bicornis* از بخش ($2n = 2x = 16$) *Eumorpha*، *O. aucheri* از بخش ($2n = 2x = 16$) *Ortholoma*، *O. suavis* از بخش ($2n = 2x = 16$) *Oxytropis* و *O. karjaginii* از بخش ($2n = 4x = 32$) *Janthina* ارائه شده است. این گزارش اولین مطالعه سیتوژنتیکی بر روی این تاکسون‌ها می‌باشد. تمام گونه‌های مورد مطالعه (به استثناء *O. karjaginii*) دیپلوئید هستند و عدد $2n = 2x = 16$ را نشان می‌دهند که با عدد کروموزومی پایه پیش بینی شده $x = 8$ مطابقت دارد. همچنین عدد کروموزومی و رفتار میوزی در *O. masanderanensis* متعلق به بخش *Ortholoma* مورد مطالعه قرار گرفت که عدد کروموزومی ($2n = 2x = 16$) را نشان داد. اگرچه جفت شدگی بی‌والان‌ها و تفکیک کروموزوم‌ها در این گونه عادی است، لیکن برخی ناهنجاری‌ها در طی میوز در این گونه مشاهده می‌شود.

INTRODUCTION

Oxytropis DC. is a genus belonging to the tribe *Astragaleae* (Ranjbar & Karamian 2003) of family *Fabaceae*. It comprises about 300 species occurring in cold mountainous regions of Europe, Asia, and North America, and is the most numerous in Central Asia (Polhill 1981). Bunge (1874) classified 181 species of *Oxytropis* into 4 subgenera and 19 sections. Boissier (1872) in *Flora Orientalis* placed 8 species into two sections, namely *Phcoxytropis* and *Euoxxytropis*, while

Vasilchenko (1984) in *Flora Iranica* placed 40 species into two subgenera, *Oxytropis* and *ptiloxxytropis*. Originally, the genus *Oxytropis* DC. was included in *Astragalus* L. in the Linnaean circumscription of the genus. Although *Oxytropis* is considered one of the closest relatives of *Astragalus* L., it was never considered in *Astragalus* after its separation made by De Candolle (1802). It differs from *Astragalus* only in keel petal (pointed vs. obtuse) and pod septum (arising from adaxial suture vs. abaxial). The recent molecular studies based on nrDNA ITS and chloroplast trnL

Intron data (Wojciechowski & al. 1999, Wojciechowski 2005), have clearly demonstrated that *Oxytropis* is monophyletic and not nested within *Astragalus* s. str., but form a separate clade within the large Astragalean clade. It has also been shown that *Oxytropis* is a sister group of *Astragalus* which has Eurasian origin. Linneaus (1753) segregated the genus *Phaca* based on inflated unilocular pods. Bunge (1868, 1869) retained *Phaca* as a subgenus of *Astragalus*. Barneby (1964) also treated it as *Phalanx*. The molecular data (Wojciechowski et al. 1999) do not support the Linneaus's concept of the genus *Phaca* and have proved that the unilocular pod has evolved many times in the genus. The genus *Oxytropis* is one of the most complex genera of this tribe and identification of the species of is very difficult and confusing. Even after the work by Vasilchenko (1988) in Flora Iranica the Iranian taxa are still in need of a new revision (Ranjbar 1999; Ranjbar 2000; Ranjbar et al. 2009). Due to their economic importance, legumes have attracted the attention of cytologists and more than 50% of their total genera are cytologically known. Most of the cytological studies in the family have been concentrated on the chromosome count and meiotic behavior in some genera (Aryavand 1983; Maassoumi 1987; Maassoumi 1989; Sheidai et al. 1996; Sheidai et al. 2000; Ghaffari 2005; Bader & Sherif 2007; Sheidai & Jalilian 2008; Sheidai et al. 2008; Ranjbar et al. 2009; Ranjbar et al. 2010), while less work have been focused on the genus *Oxytropis* (e.g. Měsíček & Soják 1969; Jurtzev 1978; Vassilczenko 1988; Jurtzev 1988; Pavlova 1989; Takhtajan 1990; Polozhij 1994). The basic chromosome number ($x = 8$) and four ploidy levels ($2n = 2x = 16$, $2n = 4x = 32$, $2n = 6x = 48$, $2n = 8x = 64$ and $2n = 12x = 96$) are present in the genus. Studies on the impact of karyotypic data on the interspecific and phylogenetic relationships and also on meiotic behavior in the genus are still limited. Also, little is known, on the genus *Oxytropis*, about the nature of genetic variability in diploid species and the taxonomic relationships of the different taxa.

MATERIALS AND METHODS

For mitosis, materials of 4 species of the Iranian *Oxytropis* species were collected, from 2000 to 2008, in different localities of Iran (Fig. 1) and pods were collected from healthy plants. Voucher specimens were deposited at the Herbarium of the Bu-Ali Sina University (BASU), Hamedan, Iran. Then, pods were left to dry at room temperature, and seeds obtained from dry pods and kept at 4 °C until used. Young root

tips were obtained from seeds germinated in Petri dishes pretreated with 0.05% colchicine for 3 h and fixed in 3: 1 ethanol: glacial acetic acid for 24 h. Root tips were hydrolyzed for 6 min in 1M HCl at 60 °C, washed briefly in dd H₂O and stained in Feulgen's solution for 1-2 h. All permanent slides were made using Venetian turpentine (Wilson 1945). The slides were examined under an Olympus BX-41 photomicroscope.

Also chromosome number and meiotic behavior were analyzed in a single population of *O. masanderanensis*. 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in Pienr's fluid containing ethanol (96%), chloroform and propionic acid, 6 : 3 : 2 (v/v/v), for 24 h at room temperature and then stored in 70% alcohol at 4 °C until used. Anthers were squashed and stained with 2% acetocarmine. All permanent slides were made using Venetian turpentine (Wilson 1945). Photographs of chromosomes were taken by Olympus BX-41 photomicroscope at initial magnification of 1000X. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

RESULTS AND DISCUSSION

Oxytropis sect. *Ortholoma* Bunge

O. suavis Boriss., Not. Syst. Leningrad 10: 80 (1947).

Iran: Khorasan, Kuchan to Sabzevar, 97 km to Sabzevar, 1492-1527 m, Ranjbar and Bayat 14186 (BASU).

According to our literature review, this is the first chromosome count for *O. suavis*. This species is close to *O. bicornis* Vassilcz., both share a basic chromosome number of $2n = 2x = 16$ (Figs. 2A C) and have similar shape and size of flowers, and also similar shape and structure of pods. However *O. suavis* differs from *O. bicornis* in possessing smaller habit, and stipule and pod indumentum.

Oxytropis sect. *Oxytropis*

O. bicornis Vassilcz., Fl. Ir. 157: 123 (1984).

Iran: Khorasan, Sabzevar to Esfaryen, Afchang village, 1492-1527 m, Ranjbar and Bayat 12871 (BASU).

This species is a herbaceous perennial. Stems have 2-4 cm length; the inflorescences bear 12 to 17 dark violet flowers and pods densely covered with short appressed hairs. This species is a semi rosette perennial and grows in clay places or clay stony zones of the sub

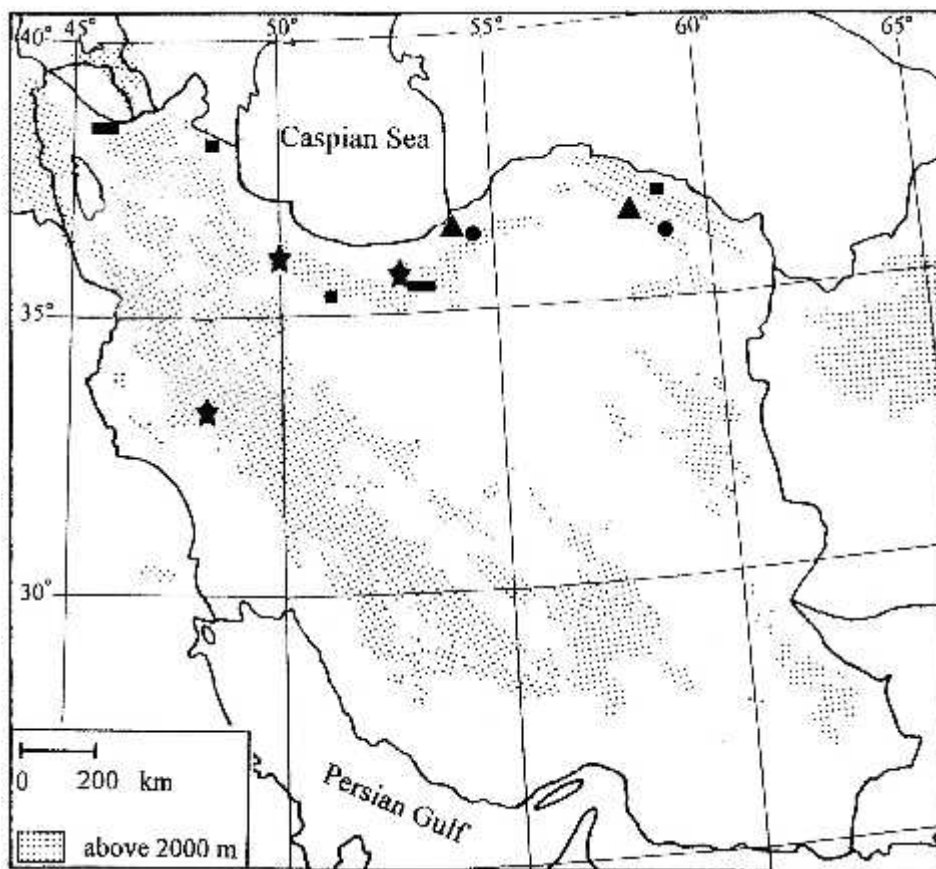


Fig. 1. Distribution map of (★) *Oxytropis masanderanensis*, (●) *O. suavis*, (■) *O. aucheri*, (▲) *O. bicornis* and (—) *O. karjaginii* in Iran.

mountainous regions in northeastern Iran. Chromosome count of $2n = 2x = 16$ (Figs. 2D & E) for this species is the first report.

***Oxytropis* sect. *Eumorpha* Bunge**

O. aucheri Boiss., Diagn. Pl. Or. Nov. Ser. 1, 2: 44 (1842).

Iran: Ardebil: Nir to Ardebil, Yamchi, 1540 m, Ranjbar, Karamian and Bayat 14187 (BASU).

This species is a small perennial. Stems are thin and reach to 10 cm. The inflorescences bear 5 to 15 small bluish flowers. It is a morphologically variable species, normally found in open fields and along roadsides, and flourishes in spring. Chromosome count of $2n = 2x = 16$ (Figs. 2F-H) for this species is the first report.

***Oxytropis* sect. *Janthina* Bunge**

O. karjaginii Grossh., Trudy Azerb. Fil. Akad. Nauk URSS 1: 54 (1933).

Iran: Azerbaijan Sharqi: Kharvanq, 1475 m, Ranjbar, Karamian and Bayat 14182 (BASU).

O. karjaginii is very common and widely distributed in Iran. This tetraploid plant is characterized by calyx teeth longer than tube, standard 12-13 mm long and leaflets in 5-7 pairs. One polyploidy level is normally found along roadsides in Kharvanq of Azarbaijan Sharqi province. It flourishes in spring and summer. Chromosome count of $2n = 4x = 32$ (Figs. 2I-K) for this species is the first report.

***O. sect. Ortholoma* Bunge**

O. masanderanensis Vassilcz., Nov. Syst. Pl. Vasc. Leningrad 17: 197 (1980).

Iran: Tehran, Tehran to Firuzkuh, 10 km after Firuzkuh, 2280 m, Ranjbar and Bayat 14182.

We collected this taxon from Tehran province. Meiosis in the species studied was regular and showed eight bivalents at first metaphase. The meiotic irregularities observed in Firuzkuh population of *O. masanderanensis* included: cytoplasmic connections, fragmented chromosomes, precocious division of centromeres and asynchronous nucleus (Figs. 3A-I).

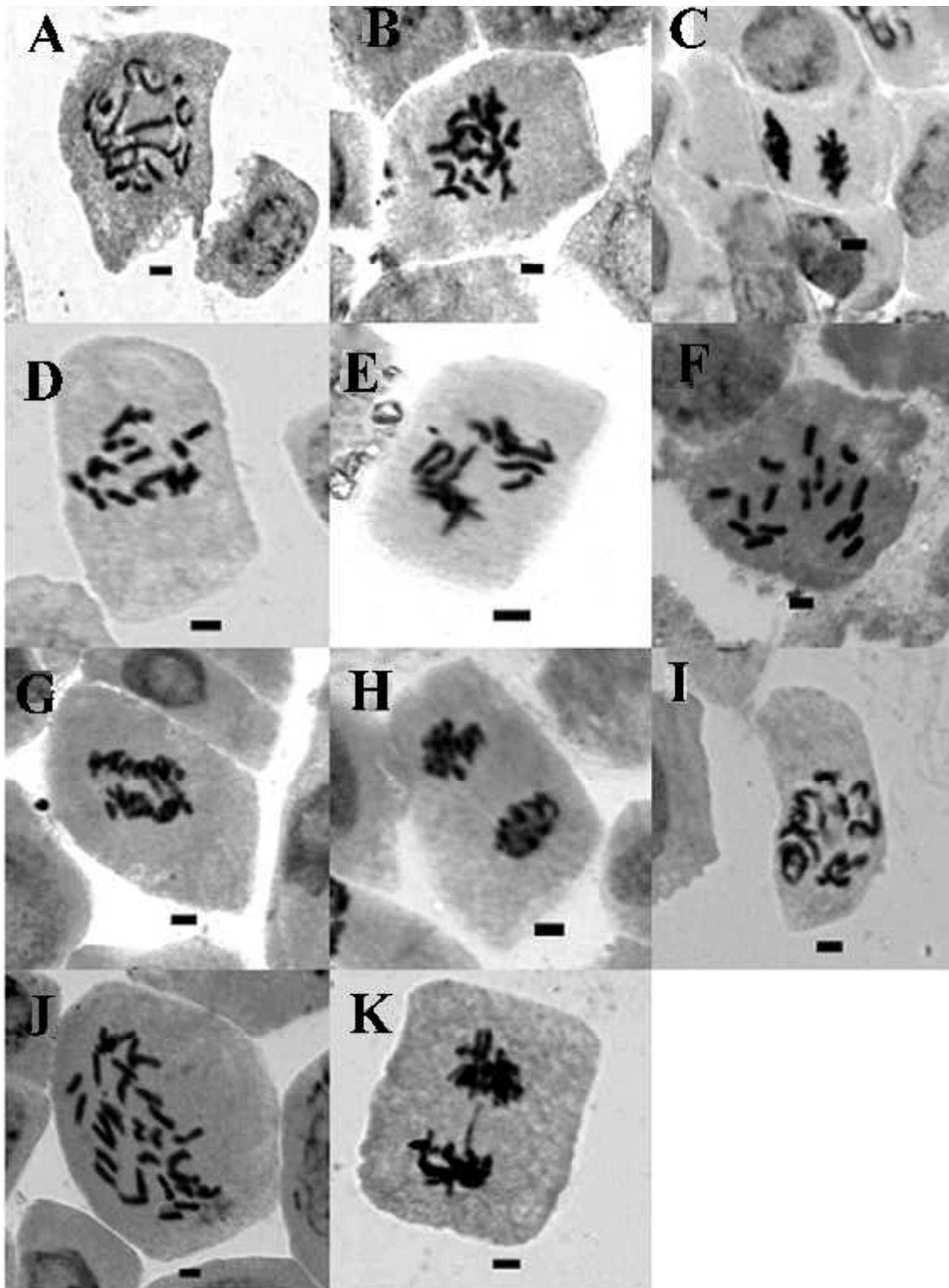


Fig. 2. Mitosis in *Oxytropis* species studied. A-C) Early prophase, prometaphase and anaphase in *O. suaveis*, respectively; D & E) Metaphase and anaphase in *O. bicornis*, respectively; F-H) Metaphase, anaphase and telophase in *O. aucheri*, respectively; I-K) Late prophase, metaphase and anaphase in *O. karjagini*, respectively. Scale bars = 3 μ m.

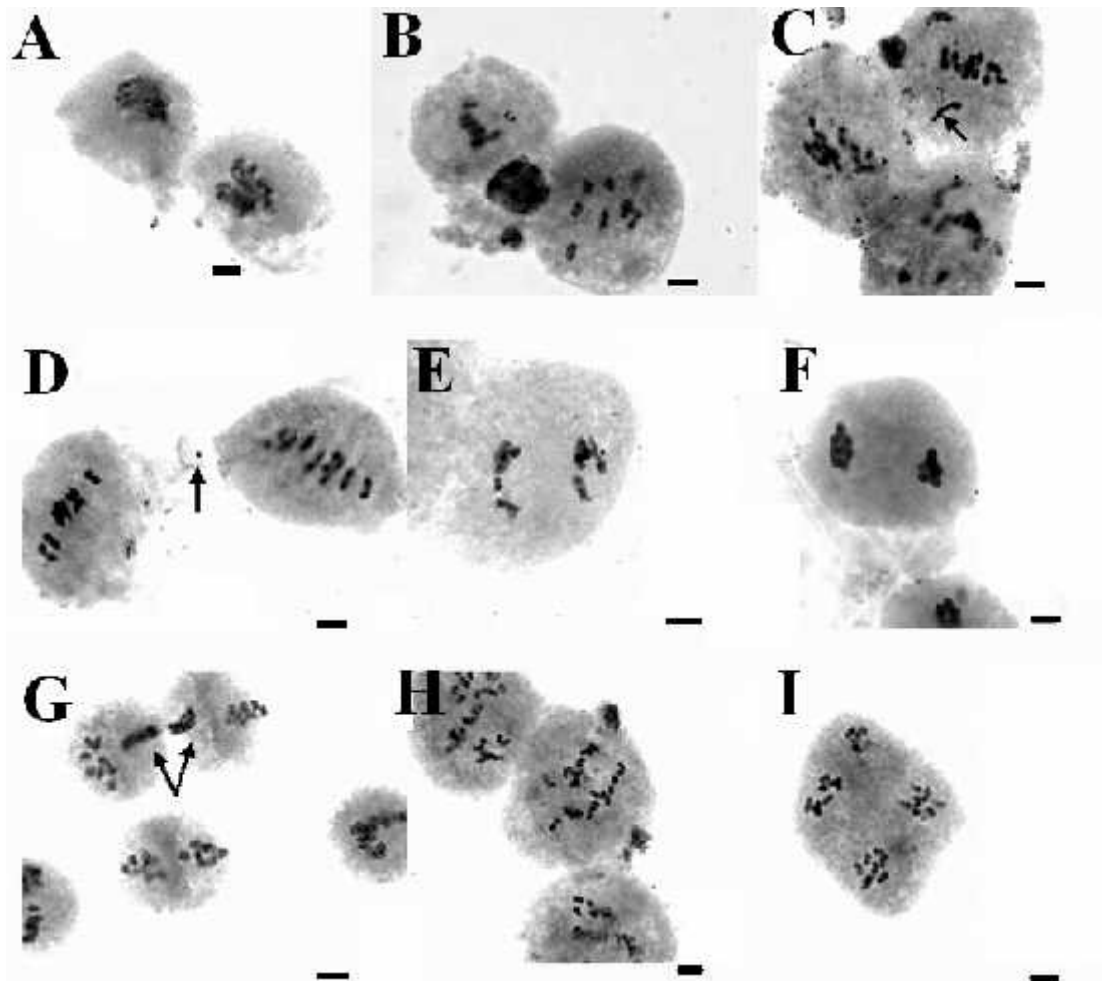


Fig. 3. Representative meiotic cells in *Oxytropis masanderanensis*. A) Diplotene; B) Diakinesis and metaphase I; C) Metaphase I with fragmented chromosomes (arrow); D) Cytoplasmic connections in metaphase I; E) Late anaphase I; F) Telophase I; G) Metaphase II (asynchronous nucleus); H) Anaphase II; I) Telophase II ($n = 8$). Scale bars = 3 μ m.

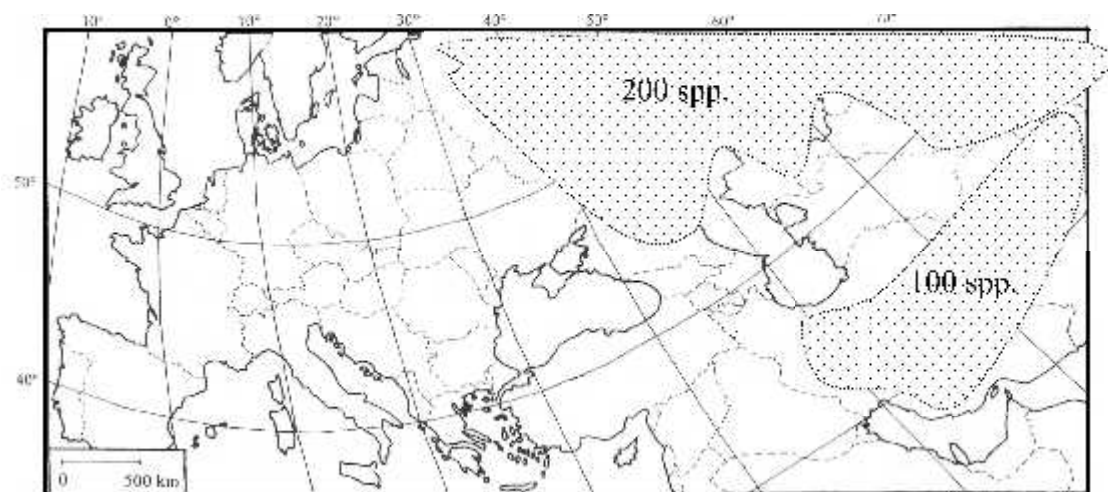


Fig. 4. Distribution map of *Oxytropis* species in Siberia, Far East, SW, C and Middle Asia.

Chromosome count of $2n = 2x = 16$ for this species is the first report.

Polyploidy, geographical distribution and mode of evolution in *Oxytropis*

The observations from the present study as well as the available data on chromosome number in the genus *Oxytropis* indicate that, among the approximately 100 species with known chromosome counts, the diploid species represent 50% of the whole and the polyploids represent 50%. Polyploids are encountered mainly in North and Middle Asia, especially in Russia, Kazakhstan, Kirgizstan, Russia-Far East and Mongolia. From this statistic it is evident that North, Middle and Southwest Asia (Fig. 4) represent the main centers of genetic diversity of *Oxytropis*. It seems that the primary centers of diversity are probably in Iran and Afghanistan with more than 100 species in low latitudes. Also a clear correlation was found between the frequency of polyploids and latitude. So the frequency of polyploids increases from the equator towards the North Pole. Polyploidy jumps dramatically from subtropical and temperate zones to cold climatic zones. The results obtained here as well as the available data on chromosome number in *Oxytropis* DC., showed an equal basic chromosome number, $x = 8$, in all species of the sections. All members of the genus *Oxytropis* are diploid with $2n = 2x = 16$ and ploidy levels of $2n = 4x = 32$, $6x = 48$, $8x = 64$, $12x = 96$ (see Jurtzev 1978; Vassilczenko 1987; Jurtzev 1988; Pavlova 1989; Takhtajan 1990; Polozhij 1994). This data indicate the important role of polyploidy in evolution and speciation. Geographic isolation has resulted in allopatric speciation, which in some groups has been complicated by polyploidy, sometimes within a single race (e.g. *O. leucantha* Jurtzev subsp. *tschukotcensis* Jurtzev with $2n = 48, 64, 96$, even within the same local population). In the *O. campestris* (L.) DC. superaggregate the lowest ploidy level in Eurasian species and races is $2n = 48$, whereas there are taxa with $2n = 16$ and 32 in the western North America. Sometimes was found the case of autopolyploidy without any visible morphological distinctions between diploid and tetraploid plants within a local population (e.g. in *O. gorodkovii* Jurtzev in the easternmost Chukotka Peninsula, $2n = 16$ and 32). In other cases polyploidy has accompanied and consolidated evolutionary adaptation to contrasting cold treeless environments (as, e.g., in the sequence: *O. sverdrupii* Lynge $2n = 48$, *O. wrangelii* Jurtzev $2n = 64$, or *O. viscida* Torr. & A. Gray aggregate (agg.) $2n = 16$ and 32 , *O. middendorffii* Trautv. agg. $2n = 48$). But there are cases of pseudo-vicarism i.e. allopatric situations,

where vicariant taxa are good species: e.g. *O. nigrescens* (Pall.) Fisch. ex DC. $2n = 16$, *O. czukotica* Jurtzev $2n = 32$ of the *O. nigrescens* (Pall.) Fisch. ex DC. agg. In general, speciation within the subsection of *Arctobia* (*O. nigrescens* (Pall.) Fisch. ex DC. agg.) has occurred on the diploid level (Yurtsev 1997). This wide geographic distribution supports the previous reports of Reese (1958), Stebbins (1972) and Ehrendorfer (1980). They have considered that polyploids have greater ability to colonize in new and wider geographic distributions than their diploid ancestors.

ACKNOWLEDGMENTS

The fieldwork in Iran was supported by grants from the Bu-Ali Sina University. The great help of Dr. E. Vitek, Dr. B. Wallnofer, Dr. W. Till and Dr. O. Sida during the first author's visit from Herbarium W, WU and PR in Vienna and Praha is much appreciated. We would like to thank the director of the herbarium Res. Inst. of For. and Rangelands (TARI) and herbarium of Ferdowsi University of Mashhad for making the herbarium facilities available for our study for the processing and loan of herbarium specimens.

REFERENCES

- Aryavand, A. 1983: IOPB Chromosome Number Reports LXXX. – Taxon 32: 504–511.
- Bader, A. & Sherif, M. S. 2007: Karyotype analysis and systematic relationships in the Egyptian *Astragalus* L. (Fabaceae). – International J. Bot. 3: 147–159.
- Barneby, R. C. 1964: Atlas of North American *Astragalus*. – Mem. New York Bot. Gard. 13: 1–1188.
- Boissier, E. 1872: *Oxytropis*. – In: Flora Orientalis, vol. 2: 491–508. Geneva, Basileae & Lugundi.
- Bunge, A. 1874: Species generis *Oxytropis* DC. Mem. Acad. Imp. Sci. Saint Petersburg, ser. 7, 22: 1–166.
- De Candolle, A. P. 1802: *Astragalologia nempe astragali, biserrulae et Oxytropidis, nec non phacae, colutae et lessertiae historia iconibus illustrata*. Parisiis J. B. Garney. 369pp.
- Ehrendorfer, F. 1980: Polyploidy and distribution. – In: Lewis, W. H. (ed.), Polyploidy: Biological relevance, pp. 45–60. Plenum Press, New York.
- Ghaffari, S. M., Hejazi, A. & Pourahmad, A. 2005: New chromosome counts in nine endemic species from Iran. – Folia Geobotanica 40: 435–440.
- Jurtzev, R. 1978: –In Flora Arctica URSS, vol. 9 (2). – Leningrad.
- 1988: A new species of the genus *Oxytropis* from the section Baicalia (Fabaceae) and the problem of

- continental relict in the flora of the Wrangel Island. Bot. Zhurn. (Leningrad), 73 (12): 1649-1661.
- Linnaeus, C. 1753: Species Plantarum. Stockholm, Sweden. pp. 755-762.
- Maassoumi, A. A. 1987: Notes on the genus *Astragalus* in Iran I, cytotaxonomic studies on some species. Iranian J. Bot. 3: 117-128.
- Maassoumi, A. A. 1989: Notes on the genus *Astragalus* in Iran IV, cytotaxonomic studies on some species. Iranian J. Bot. 4: 153-163.
- Měsíček, J. & Soják, J. 1969: Chromosome counts of some Mongolian Plants. Folia geobotanica phytotax., Praha. 4: 55-86.
- Pavlova, N. S. 1989: Fabaceae. In: Plantae Vasculares Orientis Extremi Sovietici, vol. 4. - Leningrad.
- Polhill, R. M. 1981: Galegeae. In: Polhill, R. M. & Raven, P. H. (eds.), Advances in legume systematics, part 1: 357-363. -Royal Botanical Gardens, Kew.
- Polozhij, A. V. 1994: In: Flora Sibiriae, vol. 9. Fabaceae. -Novosibirsk.
- Ranjbar, M. 1999: Some remarks on the genus *Oxytropis* (Fabaceae) from Iran. Sendtnera 6: 193-196.
- Ranjbar, M. 2000: A phenetic analysis of the species of Iranian *Oxytropis* (Fabaceae) based on morphological data. J. Sci. I. R. Iran. 11 (1): 6-10.
- Ranjbar, M. & Karamian, R. 2003: Caraganeae, a new tribe with a note on the genus *Chesneya* Lindl. ex Endl. (Fabaceae) from Flora of Iran. Thaiszia J. Bot. 13: 67-75.
- Ranjbar, M. & Bayat, S. 2009: Notes on *Oxytropis* sect. *Mesogaea* (Fabaceae) in Iran, with the description of a new species. Ann. Bot. Fennici 46: 235-238.
- Ranjbar, M. & Enayati Akmal, A. 2010: Meiotic Behavior and Chromosome Number of 5 Species of the *Astragalus* sect. *Megalocystis* Bunge (Fabaceae) in Iran. Cytologia 75(1): 49-58.
- Ranjbar, M. & Hadadi, A. 2009: Biosystematic Study of *Onobrychis viciifolia* Scop. And *Onobrychis altissima* Grossh. (Fabaceae) in Iran. Iran. J. Bot. 15(1): 85-95.
- Reese, G. 1958: Poliploidie und Verbreitung. Zeitschr. Bot. 46: 339-354.
- Sheidai, M., Iraj, Sh., Karamian, R. & Ranjbar, M. 2008: Cyto-morphological studies of the genus *Glycyrrhiza* in Iran. Cytologia 73(3): 333-339.
- Sheidai, M. & Jalilian, N. 2008: Karyotypic study of some Iranian species and populations of *Lotus* L. Acta Bot. Croat. 67: 45-52.
- Sheidai, M., Maassoumi, A. A. & Pakravan, M. 1996: Karyotypes of some *Astragalus* taxa (sect. *Xiphidium* BGE) from Iran. The nucleus 39: 111-113.
- Sheidai, M., Yazdanbakhsh, Z., Asadi, M. & Moussavi, M. 2000: Cytology and morphometric study of *Alhagi* (Leguminosae) species in Iran. Nord. J. Bot. 21: 83-91.
- Stebbins, G. L. 1972: The evolution of the grass family, pp. 1-17. In: Youngner, V. B. & Mckell, C. M. (eds.), the biology and utilization of grasses. - Academic Press, New York.
- Takhtajan, A. 1990: Nomeri chromosomatum Magnoliophytorum florum URSS, Vol. 1. Leningrad.
- Vassilczenko, I. T. 1984: *Oxytropis*. In: Rechinger, K. H. (ed.), Flora Iranica, 157, 101-164. -Akad. Druk. U. Verlags anst., Graz & Wien.
- Vassilczenko, I. T. 1988: In: Flora Partis Europaeae URSS, vol. 6. -Leningrad.
- Wilson, G. B. 1945: The Venetian turpentine mounting medium. Stain Technol. 20: 133-135.
- Wojciechowski, M. F. 2005: *Astragalus* (Fabaceae): A molecular phylogenetic perspective. Brittonia 57: 382-396.
- Wojciechowski, M. F., Sanderson, J. & Hu, J. M. 1999: Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA trnL intron data. Syst. Bot. 24: 409-437.
- Yurtsev, B. A. 1997: Analysis of evolutionary differentiation in key arctic alpine taxa: *Dryas*, *Oxytropis* sect. *Arctobia* and *Taraxacum* sect. *Arctica*. Opera Bot. 132: 27-37.