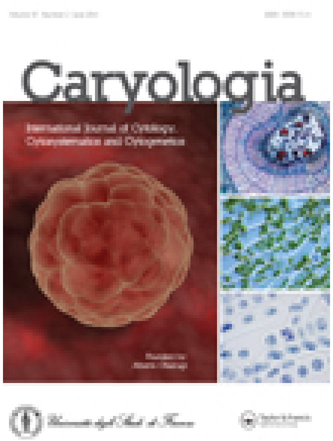

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Karyotype studies endemic plant species Astragalus sericeocanus Gontsch. (Fabaceae) around Lake Baikal, Siberia

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A study of the karyotype and chromosome of six *Astragalus sericeocanus* populations from different parts of Lake Baikal's shoreline was undertaken. Each population had the same chromosome number, $2n = 16$. The population was divided into three groups depending on the karyotype formulae $2n = 16$=8m+8sm, $2n = 16$=6m+10sm and $2n = 16$=4m+12sm. The analysis of the karyotype asymmetry indexes showed that population on Millionnyi Island had the most asymmetrical and evolutionary karyotype and the population along the River Turka had the most symmetrical karyotype in all of the populations. The plants from all studied populations have similar chromosome morphology.

Keywords: chromosome; karyotype; *Astragalus sericeocanus*; endemic plant species; population; Lake Baikal

Introduction

Astragalus L. is one of the largest genera of vascular plants in Eurasia, with an estimated 3000 species. Many species are local endemics, while relatively few are widespread, distributed mainly in the northern hemisphere of Central Asia and north-west America (Podlech 1986; Açik et al. 2004). *Astragalus* is also the largest genus of *Fabaceae* in the Baikal Siberia, where it is represented by nearly 37 species (Malyshev and Peshkova 1984). Generally, the genus *Astragalus* includes the highest number of species adapted to the mountain steppes of Baikal Siberia and contains seven (19%) endemic taxa. Although there are a lot of cytological studies on the *Astragalus*, only a few karyotypes had been studied for this genus.

Astragalus sericeocanus Gontsch. (*Fabaceae*) is a rare endemic plant of the sandy dunes of Lake Baikal. This species grows in a very limited area, generates small populations, which frequently can be represented by few individuals. It is only found between the village of Gremyachinsk and the River Turka, as well as on the islands of Yarki and Millionnyi. It is a perennial plant which flowers in July and bears fruits in August. *A. sericeocanus* plays an important ecological service by helping to protect and stabilize sandy dunes around Lake Baikal from erosion. The species belongs to the section *Cenantrum* Koch of the subsection *Semilunaria* Gontsch. *A. sericeocanus* is included in the *Astragalus penduliflorus* complex which is an assemblage of five taxa: *A. sericeocanus*, *A. penduliflorus* Lam., *A. propinquus* Schischk., *A. membranaceus* (Fisch.) Bunge, and *A. mongholicus* Bunge. All these species are similar to each other in morphology (Vydrina et al. 1994; Cherepanov 1995; Zhu 2008). Although there are many systematic, anatomic, karyological and palynological studies on the *Astragalus penduliflorus* complex, some taxonomic problems concerning section *Cenantrum* have not been resolved yet. Zhu (2008) proposed that this complex should be treated as one species. Based upon results on seed proteins and karyology (Chen and Zhu 1990), the authors also proposed *A. pendulifolius* to be a single species, comprising the subspecies *A. penduliflorus*, *A. membranaceus*, and *A. mongholicus*. However, recent revisions of species involved in this complex based on molecular phylogenetic approaches demonstrate considerable morphological and genetic diversity within each species (e.g. Xie et al. 2009; Choi and Choi 2013). The studied species *A. sericeocanus* also differs from other taxa in peptides of seed protein (Zhu and Chen 1991).

The present study details somatic chromosome characteristics of *A. sericeocanus*, including their morphology and number within the six populations from Lake Baikal (Russia).

Materials and methods

The material was collected from six populations of *A. sericeocanus* (Figure 1). These populations include the majority of all known localities for this species including an isolated population near village Turka On the eastern shore of Lake Baikal, which is situated far from the others (Sandanov et al. 2014).

(1) Yarki 1. 55°46′30.1″ N, 109°37′53.8″ E, 457 m asl, 24 August 2011. *Equisetum arvense* dominated community with patches of *Astragalus sericeocanus* and *Calamagrostis epigeios*.
(2) Yarki 2. 55°45′38.6″, 109°43′00.3″ E, 459 m asl, 25 August 2011. *Astragalus sericeocanus–Leymus secalinus* dominated community.

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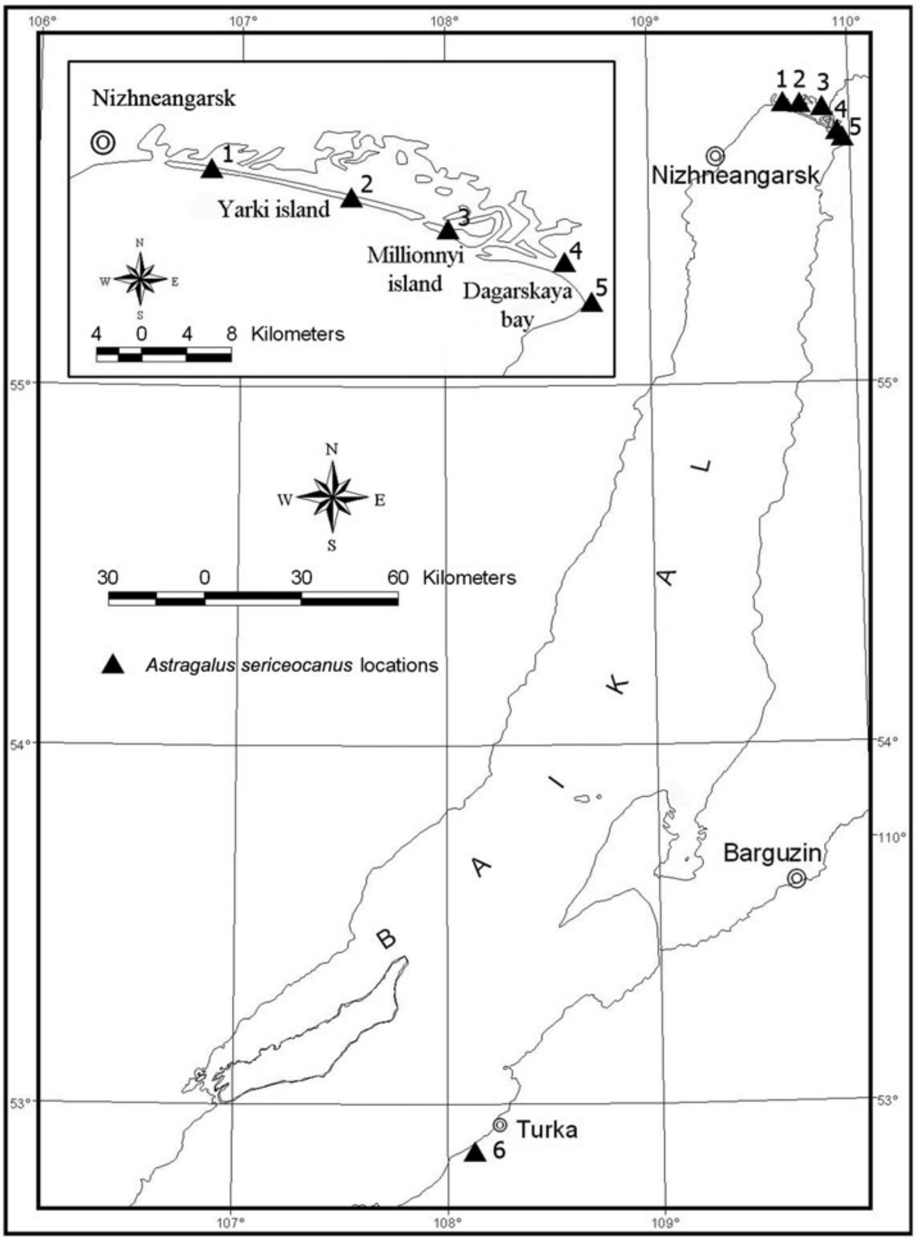

Figure 1. Distribution of populations of *Astragalus sericeocanus* around Lake Baikal. 1, Yarki 1; 2, Yarki 2; 3, Millionnyi; 4, Dagari 1; 5, Dagari 2; 6, Turka.

(3) Millionnyi. 55°43′18.4″ N, 109°46′33.4″ E, 459 m asl, 25 August 2011. *Astragalus sericeocanus–Leymus secalinus* dominated community.
(4) Dagari 1. 55°40′31.0″ N, 109°57′34.5″ E, 489 m asl, 27 August 2011. *Carex sabulosa–Astragalus sericeocanus* dominated community.
(5) Dagari 2. 55°40′30.2″ N, 109°57′34.9″ E, 467 m asl, 26 August 2011. *Festuca rubra–Astragalus sericeocanus* dominated community.
(6) Turka. 52°54′21.2″ N, 108°09′31.4″ E, 465 m asl, 5 August 2009. *Oxytropis lanata* dominated community.

Mitotic chromosomes in the somatic cells of root-tips were analyzed from pressed primary roots from germinating seeds. Seeds were soaked in disinfectant solution for 15–20 min, then they were put in Petri dishes with filter paper moistened with distilled water. Petri dishes containing seeds were kept at 25°C. Root-tips were collected when the roots reached 10–15 mm. For pretreatment, the root tips were placed into 0.2% colchicine solution and kept at a temperature of 20°C for 2 h. Then, they were transferred into Carnoy fixation solution (ethanol:glacial acetic acid, 3:1) at a temperature of 22–24°C for 24 h. Following fixation, aceto iron-hematoxylin was used for the staining stage at 100°C for 1–2 min. Root tip meristem cells were isolated on a slide and pressed. The chromosome number and total karyotype length was recorded from at least 10 cells. In addition karyotype was studied by using Axioskop-40 with an AxioCam MRc-5 high-resolution digital camera. Selected cells from individuals were photographed and the images were used for the chromosome measurements. The following measurements of each pair of chromosomes were made: s (short arm), l (long arm), c (total chromosome length). Centromere index (I_c- = s/c), relative length percentage (RL, L + S/ΣC) (Krikorian et al. 1983) and the arm ratio (r = l/s) were calculated and used to classify the chromosome according to the Levan method (Levan et al. 1964). Total form percentage (TF, 100 × ΣS/C) (Huziwara 1962) and difference of relative length (DRL, Max_{RL} – Min_{RL}) (Romero Zarco 1986) were counted. To estimate the karyotype asymmetry, three numerical parameters were used, following Romero Zarco (1986): A_1 (intrachromosomal asymmetry index = 1 – [Σ(short arm/long arm)/n]), and A_2 [interchromosomal asymmetry index = standard deviation (S)/mean length (X)]. To assess the asymmetry of each chromosome, the Mca (mean centromeric asymmetry) index was used, using the formula proposed by Peruzzi and Eroğlu (2013).

Results and discussion

The chromosome number of *A. sericeocanus* was first determined as 2*n* = 16 (specimen Turka) by Probatova et al. (2008). Later, we confirmed this and identified the chromosomal number of Turka locality as 2*n* = 16 + 1B (Konichenko et al. 2012).

In this study we determined chromosome numbers and detailed chromosome measurements of *A. sericeocanus* from different localities. Determination of chromosome numbers of plants from localities Yarki 1, Yarki 2, Millionnyi, Dagari 1 and Dagari 2 was conducted for the first time. Analysis of somatic metaphase plates showed that the chromosome numbers of the species are 2*n* = 2*x* = 16 in all investigated populations. The somatic chromosomes of *A. sericeocanus* are presented in Figure 2. Accessory chromosome B1 which was found previously in plants from the Turka was not detected.

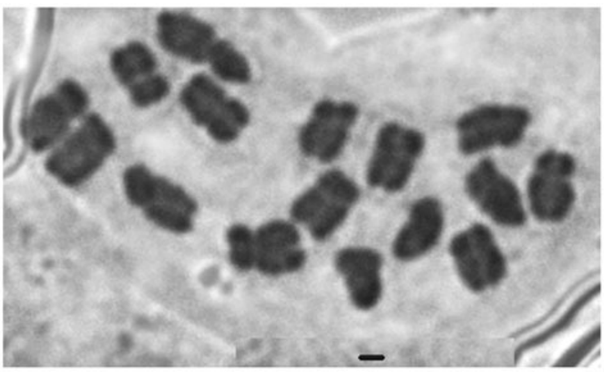

Figure 2. Chromosomes at mitosis metaphase of *Astragalus sericeocanus* (2*n* = 16). Turka population. Scale bar: 1 μm.

The basic chromosome number of *A. sericeocanus* is *x* = 8. A comparison of our results with previous data revealed that the *Astragalus* species from the Old World have a basic chromosome number of *x* = 8 (Ledingham 1960). It has been reported that a few *Astragalus* species have the basic number *x* = 7 or *x* = 6 (Badr and Sharawy 2007), but the prevalence of species with a basic number of *x* = 8 led Bard et al. (1996) to conclude that it is the primary basic number for this genus.

Karyotypes within the populations are similar in their chromosome morphology. The respective idiograms (Figure 3) are based on mean values presented in Table 1.

The populations from Yarki 1 and Yarki 2 have a formula of 2*n* = 16 = 8m + 8sm, with chromosome pairs 1, 2, 3 and 5 having centromeres in the submedian position (Table 1). The plants of populations from Dagari 1 and Dagari 2 have 2*n* = 16 = 6m + 10sm. The three pairs of chromosomes with the median centromere position are pairs 4, 6 and 8. Plants from the Turka locality have a karyotype formula of 2*n* = 16 = 6m + 10sm too, but pairs 6, 7 and 8 are metacentric. Plants from the Millionnyi population have a karyotype formula of 2*n* = 16 = 4m + 12sm, with pairs 7 and 8 metacentric. No satellites were observed in the karyotype of this species.

Based on the karyotype formula we determined three groups of populations. The first group included populations Yarki 1 and Yarki 2 with karyotype formula 2*n* = 16 = 8m + 8sm. The second group consisted of the populations Dagari 1, Dagari 2 and Turka (2*n* = 16 = 6m + 10sm). Millionnyi have different a karyotype formula (2*n* = 16 = 4m + 12sm) and are separated from all other populations.

The average length of chromosomes in the investigated populations of *A. sericeocanus* varied from 3.20 (Turka) to 3.82 μm (Yarki 1). The size of the longest chromosome pair varied from 3.96 μm in Turka to 4.86 μm in Yarki 1, while the size of shortest chromosome pair ranged from 2.49 μm in Turka to 3.39 μm in Millionnyi (Table 1).

The total haploid chromosome length of the karyotype (CL) based on the mean chromosome length ranges from 25.61 μm (Turka) to 30.55 μm (Yarki 1); 1.92–2.32 μm in the long arm and 1.27–1.46 μm in the short arm (Tables 1, 2). The total karyotype length roughly indicates the chromatin content within the studied populations.

The populations which were situated closer to each other have more similar karyotypes. For example, two populations (Yarki 1 and Yarki 2) have the same karyotype parameters, such as L (2.32 μm and 2.26 μm), C (3.82 μm and 3.78 μm), arm ratio (1.59 and 1.60) and CL (30.55 and 30.22) respectively. The karyotype parameters of the Millionnyi population were similar to those at Yarki 1 and Yarki 2 (L = 2.3 μm, C = 3.80 μm and CL = 30.43 μm). The Dagari 1 and Dagari 2 populations

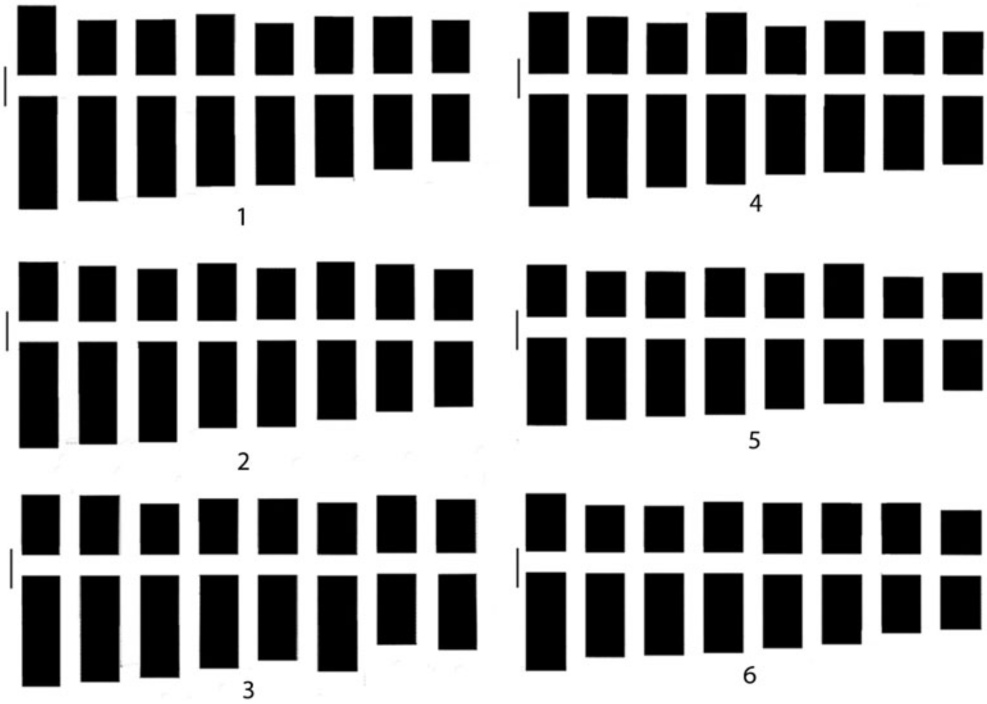

Figure 3. Haploid idiograms of *Astragalus sericeocanus* in six populations: 1, Yarki 1; 2, Yarki 2; 3, Millionnyi; 4, Dagari 1; 5, Dagari 2; 6, Turka.

have very similar parameters: long arm (2.16 μm; 2.16 μm), chromosome length (3.51 μm; 3.56 μm) and arm ratio (1.54; 1.54). The Turka population was different from the other populations in the following three values: smallest long arm 1.27 μm, chromosome length 3.20 μm, arm ratio 1.50 and total haploid chromosome length of the karyotype (CL = 25.61 μm).

The Romero Zarco (1986) intrachromosomal asymmetry index (A1) expresses the arm ratio of each pair of homologous chromosomes. The interchromosomal asymmetry index (A_2) corresponds to Pearson's coefficient of dispersion and gives an idea of the asymmetry caused by the different length of the chromosomes. By using the Romero Zarco A_1 and A_2 asymmetry indexes it is possible to determine the most asymmetric karyotype among the populations.

The results show the intrachromosomal symmetry in *A. sericeocanus* was high (Table 3). The Millionnyi population had the highest A_1 (0.41) and M_{ca} (0.26), and the lowest TF (36.64%), and therefore possessed the most asymmetrical and evolutionary karyotype. Conversely, the Turka population had the most symmetrical karyotype among the studied populations ($A_1 = 0.33$; $M_{ca} = 0.19$; TF = 39.78%). It is possible that differences in the composition of the karyotype of the Turka population are linked to its long isolation from other populations. The analysis of the karyotype asymmetry indexes for Yarki 1 and Yarki 2 populations showed a similar value A_1 (0.34;0.36), M_{ca} (0.22;0.22), TF (38.10 and 37.75%). The data obtained for populations at Dagari 1 and Dagari 2 were A_1 (0.34 0.35), M_{ca} (0.22; 0.21) and TF (39.60 and 39.22%). Based on the results of interchromosomal asymmetry with the value of highest A_2 (0.24) and DRL (5.66), populations at Yarki 1 possessed the most asymmetrical karyotype of the six populations studied.

A. sericeocanus is an endemic species observed in the vicinity of Lake Baikal and so far the number of chromosomes from most localities has not been known. Other species of the genus *Astragalus* of the subsection *Semilunaria* Gontsch. are diploid and it is likely that all species of this subsection in the Eurasian geographic range are uniform in ploidity-diploids (Majovsky et al. 1996). The data obtained for *A. sericeocanus* as the diploid species with $2n = 2x = 16$ supported the results of other research concerning chromosomal study of *Astragalus* section *Cenantrum* (Gangrong 2003). Kim et al. (2006) showed that *A. membranaceus* Bunge has a karyotypic formula of $2n = 16 = 4$m (3, 4, 6 and 7 pairs) + 4sm, *A. membranaceus* var. *alpinus* Nakai has karyotypic formula $2n = 16 = 2$m (4 and 8 pairs) + 6sm and *A. mongolicus* Bunge has $2n = 16 = 3$m (6, 7 and 8 pairs) + 5sm. Zhu (1996) described the karyotypic formula for *Astragalus minhensis* X. Y. Zhu & C. J. Chen as $2n = 16 = 8$m (2SAT) + 8sm.

The findings of the present study reveal that the karyotype formula of specimens collected from different localities may be different. The results of this study may serve to clarify the taxonomic position of *A. sericeocanus* in *Astragalus penduliflorus* Lam. complex. We hope that this study will contribute to the future karyological studies of the genus *Astragalus.*

Table 1. Karyomorphological parameters of *A. sericeocanus* in six populations.

Locality	Pair no.	S (µm)	L (µm)	C (µm)	R	RL (%)	CI	M_{ca}	Type
Yarki 1	1	1.68 ± 0.05	2.86 ± 0.09	4.86 ± 0.14	1.70	15.91	35.89	0.25	sm
	2	1.38 ± 0.02	2.70 ± 0.05	4.10 ± 0.05	1.95	13.42	33.65	0.32	sm
	3	1.43 ± 0.04	2.57 ± 0.07	4.02 ± 0.09	1.79	13.16	35.57	0.28	sm
	4	1.56 ± 0.04	2.27 ± 0.04	3.85 ± 0.06	1.45	12.6	40.51	0.18	m
	5	1.29 ± 0.02	2.36 ± 0.04	3.64 ± 0.05	1.82	11.91	35.43	0.29	sm
	6	1.46 ± 0.06	2.08 ± 0.04	3.59 ± 0.06	1.42	11.75	40.66	0.17	m
	7	1.47 ± 0.04	1.93 ± 0.07	3.36 ± 0.08	1.31	11	43.75	0.13	m
	8	1.37 ± 0.04	1.75 ± 0.04	3.13 ± 0.08	1.27	10.25	43.76	0.12	m
Yarki 2	1	1.51 ± 0.05	2.76 ± 0.06	4.57 ± 0.12	1.82	15.12	33.04	0.29	sm
	2	1.39 ± 0.04	2.61 ± 0.05	4.14 ± 0.09	1.87	13.69	33.57	0.30	sm
	3	1.34 ± 0.06	2.56 ± 0.05	4.04 ± 0.08	1.91	13.37	33.16	0.31	sm
	4	1.51 ± 0.05	2.26 ± 0.04	3.91 ± 0.11	1.49	12.94	40.10	0.19	m
	5	1.33 ± 0.06	2.28 ± 0.06	3.61 ± 0.08	1.71	11.95	36.84	0.26	sm
	6	1.48 ± 0.07	2.03 ± 0.04	3.58 ± 0.07	1.37	11.85	41.34	0.15	m
	7	1.41 ± 0.04	1.87 ± 0.06	3.29 ± 0.09	1.32	10.89	42.85	0.14	m
	8	1.32 ± 0.06	1.70 ± 0.08	3.08 ± 0.12	1.28	10.19	42.85	0.12	m
Millionnyi	1	1.56 ± 0.06	2.84 ± 0.05	4.51 ± 0.07	1.82	14.86	34.06	0.29	sm
	2	1.50 ± 0.05	2.69 ± 0.13	4.38 ± 0.06	1.79	14.44	34.24	0.35	sm
	3	1.27 ± 0.12	2.61 ± 0.13	3.89 ± 0.05	2.05	12.82	32.64	0.34	sm
	4	1.38 ± 0.02	2.40 ± 0.12	3.68 ± 0.07	1.73	12.13	37.50	0.26	sm
	5	1.39 ± 0.11	2.20 ± 0.11	3.59 ± 0.09	1.58	11.83	39.94	0.22	sm
	6	1.17 ± 0.04	2.42 ± 0.12	3.58 ± 0.08	2.07	11.8	32.68	0.35	sm
	7	1.50 ± 0.07	1.82 ± 0.10	3.41 ± 0.05	1.21	11.24	43.60	0.09	m
	8	1.38 ± 0.01	2.08 ± 0.08	3.39 ± 0.05	1.50	11.17	40.70	0.20	m
Dagari 1	1	1.56 ± 0.06	2.76 ± 0.04	4.33 ± 0.05	1.76	15.39	36.02	0.27	sm
	2	1.41 ± 0.02	2.59 ± 0.08	3.99 ± 0.07	1.83	14.18	35.33	0.29	sm
	3	1.36 ± 0.02	2.38 ± 0.06	3.69 ± 0.09	1.75	13.11	36.85	0.27	sm
	4	1.52 ± 0.02	2.19 ± 0.08	3.66 ± 0.09	1.50	13.01	40.42	0.18	m
	5	1.26 ± 0.06	2.04 ± 0.03	3.33 ± 0.07	1.61	11.83	37.83	0.23	sm
	6	1.33 ± 0.03	1.98 ± 0.03	3.27 ± 0.06	1.48	11.62	40.67	0.20	m
	7	1.12 ± 0.02	1.87 ± 0.04	3.01 ± 0.06	1.66	10.7	37.20	0.25	sm
	8	1.56 ± 0.06	1.68 ± 0.02	2.80 ± 0.03	1.48	9.95	40.35	0.04	m
Dagari 2	1	1.56 ± 0.04	2.62 ± 0.04	4.18 ± 0.06	1.67	14.67	37.06	0.25	sm
	2	1.38 ± 0.04	2.43 ± 0.04	3.82 ± 0.05	1.76	13.4	36.12	0.27	sm
	3	1.33 ± 0.01	2.40 ± 0.01	3.73 ± 0.02	1.80	13.09	35.75	0.28	sm
	4	1.50 ± 0.02	2.23 ± 0.03	3.72 ± 0.03	1.48	13.05	40.32	0.20	m
	5	1.36 ± 0.02	2.15 ± 0.03	3.51 ± 0.05	1.58	12.32	38.74	0.22	sm
	6	1.50 ± 0.02	2.00 ± 0.02	3.49 ± 0.04	1.33	12.25	42.85	0.14	m
	7	1.26 ± 0.02	1.88 ± 0.03	3.17 ± 0.02	1.49	11.12	39.74	0.19	sm
	8	1.29 ± 0.02	1.59 ± 0.01	2.88 ± 0.02	1.23	10.11	44.79	0.10	m
Turka	1	1.47 ± 0.02	2.47 ± 0.06	3.96 ± 0.05	1.68	15.67	37.12	0.25	sm
	2	1.19 ± 0.05	2.16 ± 0.09	3.39 ± 0.11	1.81	13.42	35.10	0.28	sm
	3	1.20 ± 0.05	2.12 ± 0.05	3.32 ± 0.10	1.76	13.14	36.14	0.27	sm
	4	1.29 ± 0.06	2.03 ± 0.05	3.31 ± 0.07	1.57	13.1	38.37	0.22	sm
	5	1.29 ± 0.05	1.89 ± 0.06	3.18 ± 0.08	1.46	12.58	31.44	0.18	sm
	6	1.29 ± 0.05	1.81 ± 0.06	3.11 ± 0.07	1.40	12.31	41.47	0.16	m
	7	1.33 ± 0.05	1.51 ± 0.02	2.85 ± 0.07	1.13	11.28	46.66	0.06	m
	8	1.13 ± 0.12	1.37 ± 0.03	2.49 ± 0.02	1.21	9.85	45.38	0.09	m

Abbreviations: S, short arm length; L, long arm length; C, chromosome length; R, arm ratio – long arm/short arm; RL, relative length; CI, centromeric index coefficient; M_{ca}, mean centromeric asymmetry.

Table 2. Karyotype characteristics of six populations of *A. sericeocanus* ($2n = 16$).

Locality	Karyotype formula (2*n*)	S (µm)	L (µm)	C (µm)	R	RL (%)	CI	CL (µm)
Yarki 1	8m + 8sm	1.46 ± 0.04	2.32 ± 0.38	3.82 ± 0.19	1.59 ± 0.09	12.50 ± 0.61	38.65 ± 1.41	30.55
Yarki 2	8m + 8sm	1.41 ± 0.03	2.26 ± 0.13	3.78 ± 0.17	1.60 ± 0.09	12.57 ± 0.57	37.97 ± 1.53	30.22
Millionnyi	4m + 12sm	1.39 ± 0.05	2.38 ± 0.12	3.80 ± 0.15	1.72 ± 0.10	12.50 ± 0.50	36.92 ± 1.46	30.43
Dagari 1	6m + 10sm	1.39 ± 0.06	2.16 ± 0.12	3.51 ± 0.18	1.54 ± 0.07	12.47 ± 0.64	39.42 ± 1.13	28.08
Dagari 2	6m + 10sm	1.40 ± 0.04	2.16 ± 0.12	3.56 ± 0.14	1.54 ± 0.07	12.50 ± 0.50	39.42 ± 1.13	28.50
Turka	6m + 10sm	1.27 ± 0.04	1.92 ± 0.13	3.20 ± 0.15	1.50 ± 0.09	12.67 ± 0.60	38.96 ± 1.84	25.61

Abbreviations: S, short arm length; L, long arm length; C, chromosome length; R, arm ratio – long arm/short arm; RL, relative length; CI, centromeric index coefficient; CL, total haploid chromosome length.

Table 3. Mean comparison of karyotype parameters on different *Astragalus sericeocanus* populations.

Locality	DRL	TF (%)	A_1	A_2
Yarki 1	5.66	38.10	0.34	0.24
Yarki 2	4.93	37.35	0.36	0.25
Millionnyi	3.69	36.64	0.41	0.16
Dagari 1	5.44	39.60	0.34	0.13
Dagari 2	4.56	39.22	0.35	0.13
Turka	5.82	39.78	0.33	0.23

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