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Notes on karyomorphology of *Melilotus officinalis* populations in Bulgaria

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Abstract — Karyological study was conducted on 9 populations of *Melilotus officinalis* (L.) Pall. collected from different floristic regions in Bulgaria. The karyotypes of the investigated populations are similar in their chromosomal morphology consisting of metacentric and submetacentric chromosomes. Three cytotypes are delimited on the base of differences in the composition of the karyotypes and marked chromosomal differences as to length and morphological features. A greater difference in the composition of the karyotype in one of the populations is connected with deviations in its macromorphology which are taxonomically presented by separation of a variety *arenaria*. This taxon is a local endemic and its populations should be placed under control and protection. From a taxonomic point of view the minor differences in the karyotypes established give reasons to consider these cytotypes as infraspecific taxa. The chromosomal polymorphism testifies the susceptibility of the karyotype to the environmental factors resulting in genome modifications.

Key words: chromosome numbers, karyotypes, *Melilotus officinalis*, populations

INTRODUCTION

The species *Melilotus officinalis* (L.) Pall. is related to section *Coelorytis* Ser. of genus *Melilotus* (Shulz 1901). It is one of the most widely distributed species. Meusel *et al.* (1965) determined it as an European-caucasian-centralasiatic-anatolian-siberian-westnorthafrican geoelement.

This taxon is distributed mozaically in Bulgaria on humid places as a low competitive adventive species or as a ruderal plant on disturbed terrains, along roads and settlements in the lowlands and foothills up to 800 m a.s.l. (Kozuharov 1976, 1992).

The species is of agricultural and medicinal importance. In this aspect the study of its karyotype provides additional data on the variation pattern within the populations for the solution of taxonomical problems and successful attempts for selection as well.

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MATERIALS AND METHODS

The karyological study was conducted on 9 populations of *Melilotus officinalis* (A...I) collected from different floristic regions in Bulgaria indicated on an UTM Grid map of Bulgaria (Scale 1: 1500000) (Fig. 1). The vaucher specimens are deposited in the Herbarium of Sofia University St. Kliment Ohridski, Department of Botany (SO).

The karyotypes are investigated on metaphase plates (five for each population) prepared from root meristem of germinating seeds, treated in hot hydrolysis, stained in chematoxilin after Pearse (1960). The chromosomal type is determined after the centromere index I^c=s/s+l (%) concordant with the classification proposed by Grif and Agapova (1986) (metacentric (m) I^c = 50.0-40.1 %; submetacentric (sm) - I^c = 40.0-30.1 %; intercentric (i) - I^c = 30.0-20.1 %; subacrocentric (sa) - I^c = 20.0-10.1 %; acrocentric (a) - I^c = 10.0-0.1 %; telocentric (t) - I^c = 0%).

The karyotypes and their composition are shown on Fig. 2 and Table 1. The measurements of the short (s) and long (l) chromosome arms, and the absolute chromosome length (s+l) were performed on an Amplival (Carl-Zeiss Yena) microscope at magnification 2000×. The idiograms are constructed for each standard karyotype (Fig. 3, Table 2).

The method of Romero Zarco (1986) was applied to describe the karyotype asymmetry. The latter is expressed by two numerical parameters A_1 (intrachromosomal asymmetry index) and A_2 (interchromosomal asymmetry index). Karyotype asymmetry for the relations between the chromosome arms has been esti-

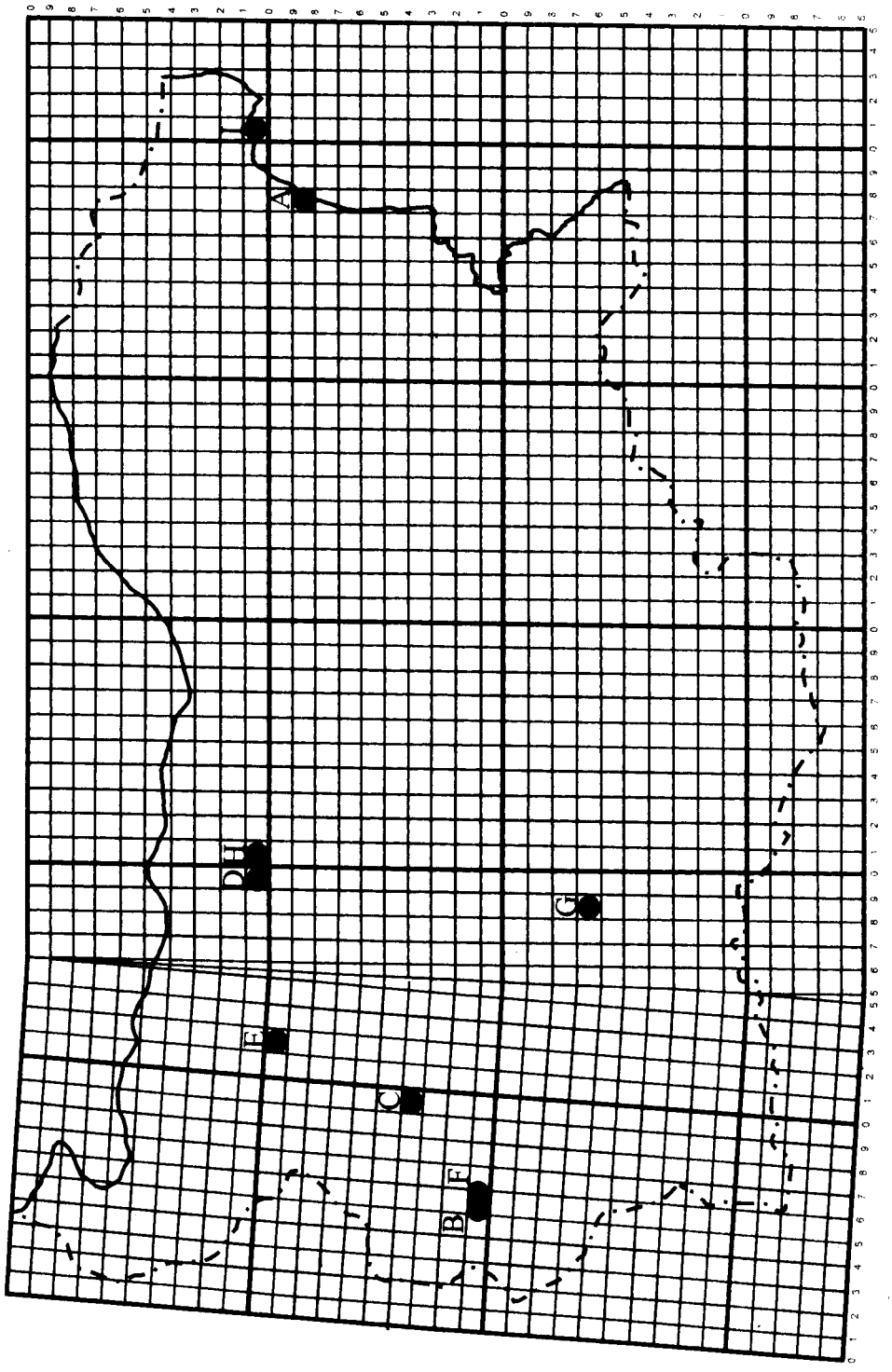

Fig. 1 — An UTM Grid map of Bulgaria showing the location of investigated populations mentioned in the text and Table 1 (Scale bar 1: 1500000). A — Varna; B — Kalista; C — Sofia; D — Dolni Dabnik; E — Elin Pelin; F — Lobosh; G — Ognjanovo; H — Pleven; I — Kavarna.

Table 1 — Material source of the populations of *Melilotus officinalis* studied and data on the karyotypes

population	Taxon	2n	M	SM	I	M^{sat}	SM^{sat}	B	Xmax:Xmin
A	Varna SO 100300, NH-78	16	10	4	—	—	2	—	1.8: 1
B	Kalista SO 100593, FN-50	16	10	4	—	—	2	—	1.9: 1
C	Sofia SO 100592, FN-93	16	10	4	—	—	2	—	1.5: 1
D	Dolny Dabnik SO 101938, KJ-90	16	10	4	—	—	2	—	1.7: 1
E	Elin Pelin SO 100594, GN-19	16	10	4	—	2	—	—	1.5: 1
F	Lobosh SO 100590, FN-50	16	10	4	—	2	—	—	1.7: 1
G	Ognjanovo SO 100578, KG-86	16	10	4	—	2	—	—	1.7: 1
H	Pleven SO 100589, LJ-00	16	10	4	—	—	2	2	1.5: 1
I	Kavarna SO 100591, PJ-00	16	8	6	2	2	—	—	1.7: 1

Table 2 — Data on the chromosomes of the three standard karyotypes of *Melilotus officinalis*.

Standard karyotypes	Populations (N°)	Pair N°	s+l, (μm)	I^c, %	Chromosome type
I. 2n = 2x = 10m +4sm + $2sm^{sat}$ = 16	A, B, C, D, H	1	2.4+2.5=4.9	49.0	m
		2	2.1+2.4=4.5	46.7	m
		3	1.7+1.8=3.5	48.6	m
		4	1.6+1.7=3.3	48.5	m
		5	1.5+1.6=3.1	48.4	m
		6	1.5+3.0=4.5	33.3	sm
		7	1.4+2.7=4.1	34.1	sm
		8 (sat)	1.4+2.7=4.1	34.1	sm
II. 2n = 2x = 10m +4sm + $2m^{sat}$ = 16	E, F, G	1	2.1+2.3=4.4	47.8	m
		2	2.0+2.4=4.4	45.5	m
		3	1.6+2.3=3.9	41.0	m
		4	1.6+1.7=3.3	48.5	m
		5	1.4+1.4=2.8	50.0	m
		6	1.5+2.8=4.6	32.6	sm
		7	1.3+2.5=3.8	34.2	sm
		8 (sat)	1.3+1.5=2.8	46.4	m
III. 2n = 2x = 8m + 4sm + 2i + $2m^{sat}$ = 16	I	1	2.0+2.4=4.4	45.5	m
		2	1.6+2.3=3.9	41.0	m
		3	1.6+2.0=3.6	44.4	m
		4	1.6+1.6=3.2	50.0	m
		5	1.6+3.2=4.8	33.3	sm
		6	1.0+2.3=3.3	30.3	sm
		7	1.2+3.2=4.4	27.3	i
		8 (sat)	1.6+1.6=3.2	50.0	m

mated for every sample using the following equation: A_1=1-(Sb_i / B_i) / n, where A_1 is the intrachromosomal asymmetry index, ranging from zero to one; n – number of homologous chromosome pairs; b_i – average length for short arms in every homologous chromosome pair; B_i – average length for long arms in every homologous chromosome pair. Karyotype asymmetry due to relations between size of different chromosomes has been estimated using Pearson's dispersion coefficient A_2=S / ξ, where S – standard deviation and ξ – the mean of chromosome length for each sample. The interchromosomal asymmetry index A_2 is independent from the chromosome size, chromosome number and has no units. The results are shown on Fig. 4 and Table 3. This method is appropriate when there are only slight differences in the karyotype asymmetry.

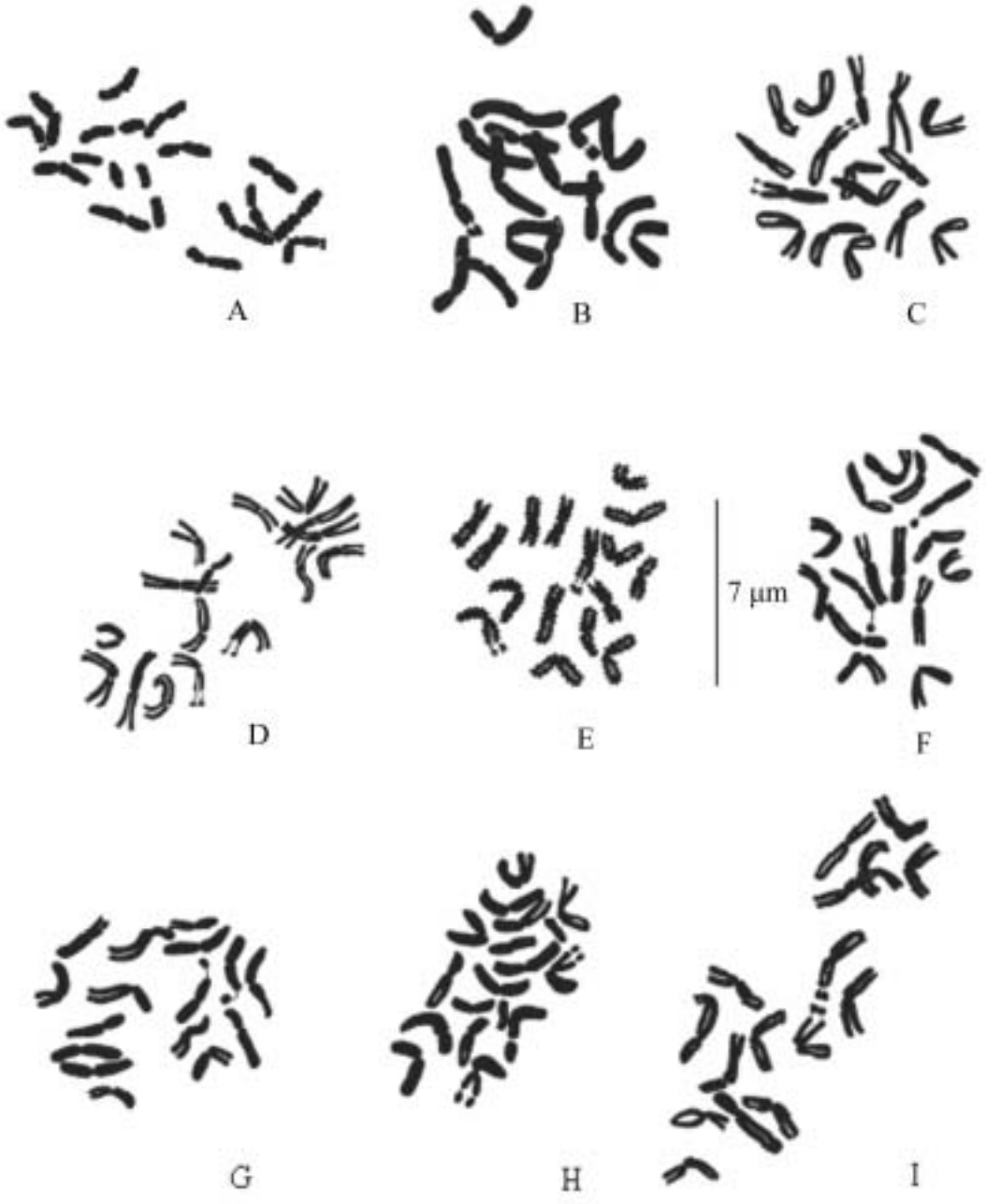

Fig. 2 — Somatic metaphases of the investigated populations (scale bar 7μm).

RESULTS AND DISCUSSION

All investigated populations of *M. officinalis* are diploids (2n=16) and confirm previous results (KITA 1966; FEDOROV 1969; GOLDBLATT 1981, 1988; GOLDBLATT and JOHNSON 1990, 1991, 1994, 1996; S OPOVA and SEKOVSKI 1982).

The analysis shows that the standard karyotype is characterized by a relatively homogenous composition. Following the classification of the chromosomal type proposed by GRIF and AGAPOVA (1986) two types of chromosomes were found: metacentric and submetacentric, excluding population I where there is a pair of intercentric chromosomes as well. Some differences are observed in the composition of the karyotypes investigated (Table 1) that can be summarized as follows:

1) The composition of the karyotypes gives ground to distinguish three cytotypes (Fig. 3, Table 2):

A) 2n= 10m + 4sm + $2sm^{sat}$ (populations A, B, C, D and H)

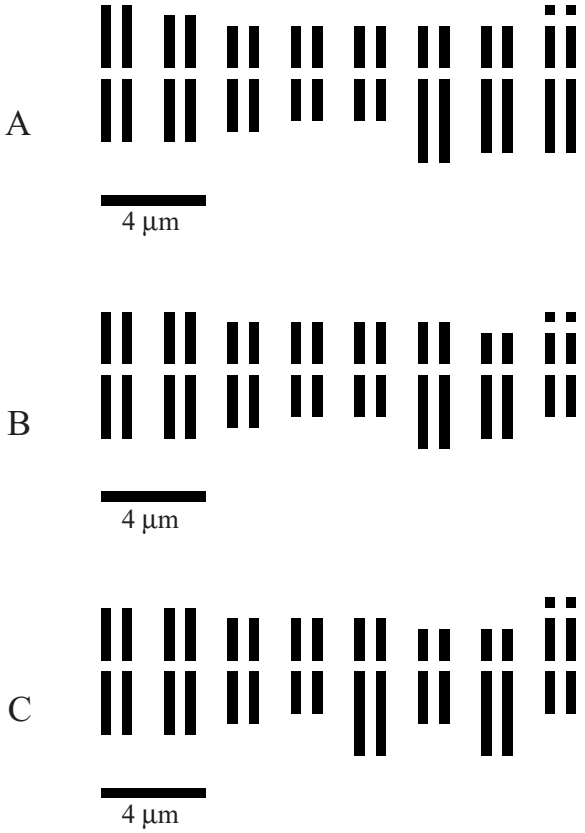

Fig. 3 — Idiograms of the three standard karyotypes (scale bar 4μm).

B) 2n= 10m + 4sm + $2m^{sat}$ (populations E, F and G)

C) 2n= 8m + 4sm + 2i + $2m^{sat}$ (population I).

2) All karyotypes posses a pair of chromosomes with satellites. At 5 of the karyotypes (A, B, C, D and H) the satellites are attached to the chromosomes of SM-type and at the rest – to the chromosomes of M-type. Satellites with larger dimensions are more frequently observed while small, spherical satellites are found only in 3 populations (A, C and F).

3) Heteromorphic chromosome pairs are established in populations C, D, F, G and H. These chromosomal aberrations are observed mainly at chromosomes with satellites, with the exception at one pair of metacentric chromosome in populations D and F.

4) The dimensions of the chromosomes from different localities do not show considerable difference. The ratio X_{max}:X_{min} ranges from 1.5: 1 (C, E, H) to 1.9: 1 (B). The length of the shortest chromosomes is 2.2 – 3.2 μm, and of the longest ones is 4.4 – 5.6 μm.

5) Additional B-chromosomes are established only in population H. The morphology of these chromosomes is unclear and their length is 1.6 μm.

6) The most symmetrical karyotypes are those of populations A, C and H. The most clearly asymmetrical karyotype is found in population I.

The application of the method of Romero Zarco (1986) allows to define clearly the asymmetry of the karyotypes within the investigated populations of *M. officinalis* (Fig. 4).

The analysis of the karyotypes indicates variation of the coefficients A_1 and A_2 with values for A_1 between 0.18 and 0.31, and for A_2 between 0.14 and 0.22 (Table 3). The most symmetrical karyotype (minimal value for A_1) is found in populations A and C while the most asymmetrical karyotype (maximal value for A_1) is found in population I.

As a result of the karyotype variation established three groups of similarity could be distinguished:

Group I – populations A, B, C, D, F and H. The coefficient A_1 ranges between 0.23 and 0.31, while A_2 – between 0.14 and 0.18. The values of A_1 and A_2 are the lowest in populations A and C, and the highest in population B. The latter population stands aside of the group, most probably due to the high absolute length of the karyotype. Population F is a linkage between Group I and Group II as its A_1 and A_2 values are 0.17 and 0.24, respectively.

Group II – populations E and G. The coefficient A_1 ranges between 0.20 and 0.21, while A_2 – between 0.18 and 0.20. In both populations are established heteromorphic chromosome pairs.

Table 3 — Data about total length of chromosomes (X), mean of chromosome length for each sample (ξ), standard deviation (S), A_1 - intrachromosomal asymmetry index, A_2 - interchromosomal asymmetry index for the populations of *Melilotus officinalis*

Population, N°	X, μm	ξ, μm	S (standard deviation)	A_1	A_2
A	67.20	4.200	0.588	0.232	0.140
B	66.68	4.167	0.743	0.305	0.178
C	61.08	3.817	0.538	0.2328	0.141
D	60.80	3.800	0.676	0.278	0.178
E	66.12	4.133	0.858	0.200	0.208
F	60.00	3.750	0.648	0.242	0.173
G	58.12	3.508	0.692	0.181	0.197
H	57.88	3.617	0.526	0.270	0.145
I	57.88	3.625	0.770	0.283	0.213

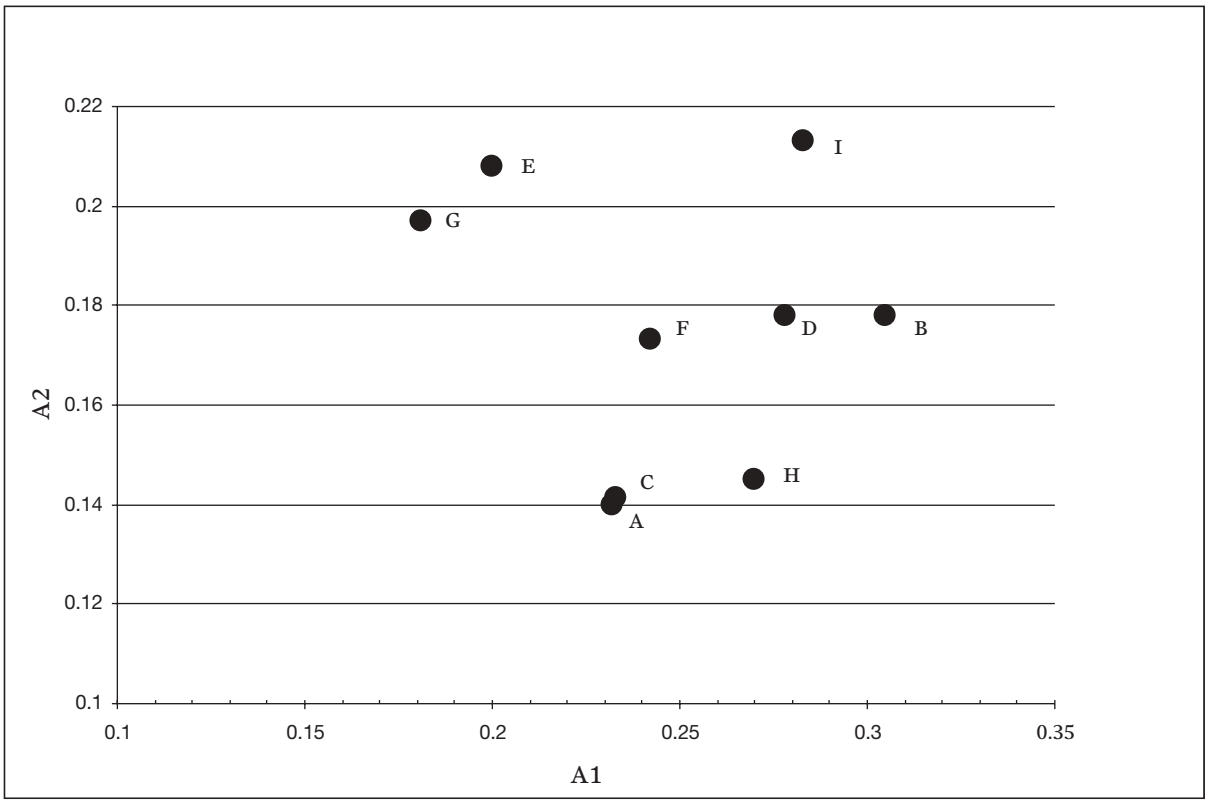

Fig. 4 — Diagram of the values of A_1 and A_2 showing the karyotype asymmetry of the *Melilotus officinalis* populations examined.

Group III – population I. This population differs by the high values of both A_1 and A_2, 0.28 and 0.22 respectively.

Group I and Group II are distinguished on the base of differences caused by the chromosome asymmetry. Cytotype A corresponds completely to the data reported by KITA (1966) for this species. The second cytotypes differs from the first one by the type of chromosomes with satellites. The observed variation in the populations of *M. officinalis* in respect to stem, height, leaf size and shape, branching, inflorescence length and thickness, and the number of flowers as well, is probably of polyallel nature (KOZUHAROV 1976).

Obviously, the most asymmetrical karyotype belongs to population I. The placement of this population in a separate group correlates well with the observed differences in the karyotype composition and the presence of a pair of intercentric chromosomes, and a chromosome pair between submetacentric and intercentric type. The karyotype specificity can be linked with deviations in the population macromorphology – smaller plants, shorter and not numerous inflorescences, hairs on the lower leaflet surface, and larger legumes. This macromorphological characters allowed HAYEK (1927) to distinguish the variety *arenaria*. The karyological results confirm the presence of variety *arenaria* in the Bulgarian flora as a separate taxon as it was stated by KOZUHAROV (1976).

This taxon is a local endemic and its populations should be placed under control and protection. The List of Medicinal Plants in Bulgaria has to be corrected by excluding this variety in order to preserve the genetic richness of the species.

CONCLUSIONS

The establishment of heteromorphic chromosome pairs in the karyotypes of *M. officinalis* testifies to significant chromosomal rearrangements in the course of the evolutionary process. The find of B-chromosomes for the first time in the karyotype of *M. officinalis* (population E) could be explained by the variety of habitats and ecological conditions to which the species is successfully adapted (BATTAGLIA, 1964).

The variations in chromosome size and type of a given species observed in natural conditions, and the changes in the structural type of chromosomes in the evolutionary process indicate that the karyotype is most susceptible to the environmental factors result-

ing in genotype changes. The karyotype symmetry is a reflection of the mutation process. From a taxonomic point of view the minor differences in the karyotypes established give reasons to consider these cytotypes as infraspecific taxa.

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