Chemotaxonomic study of some species of genus *Genista* (*Fabaceae*). Cluster analysis

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Abstract. Correlations in the alkaloid composition of ten species of genus *Genista* distributed in Bulgaria have been established by means of cluster analysis. Certain degree of similarity and affinity has been determined between the species and the sections they belong to *G. tinctoria*, *G. ovata* and *G. depressa*, on the one hand, and *G. rumelica* and *G. lydia*, on the other, are well differentiated within the limits of section *Genista*.

Key words: alkaloids, cluster analysis, *Genista*

Introduction

The taxonomic status of species is mainly determined by their morphological and cytological characteristics. In the last several decades secondary metabolites have been successfully used in taxonomy as taxonomic markers. They have made it possible to trace out better the affinities and evolution within the different taxonomic groups.

Of the secondary metabolites, alkaloids are among the most frequently used taxonomic markers. For the alkaloid-bearing representatives of family *Fabaceae* these are Quinolizidine alkaloids. The results of investigations into the alkaloid composition of the species of tribe *Genisteae* in recent years (Christov & al. 1990; Christov & al. 1991; Christov 1992) have made possible their use in taxonomy.

The purpose of the present study is to establish the degree of similarity of the investigated species of genus *Genista* and the evolutionary trends within the sections they belong to by subjecting their alkaloid composition to cluster analysis.

Material and methods

The alkaloid composition of 10 species of genus *Genista* growing spontaneously in Bulgaria is presented. They were analysed by the cluster method based on the so-called measure of disagreement between the species. Two of the investigated species, *G. rumelica* and *G. subcapitata*, are endemic to the Balkan Peninsula. Two other species have not been included owing to the fact that four populations of *G januensis* practically do not contain alkaloids, while *G. germanica* was not found during the period of research.

The investigated taxa are included in the taxonomical schemes of Gibbs (1968) and Kuzmanov (1976) (Table 1).

Table 1. Taxonomical schemes of *Genista* according to Gibbs (1968) and Kuzmanov (1976).

Section	Gibbs	Kuzmanov
Genista	G. *tinctoria* L. (incl. *G. ovata* Waldst. & Kit., *G. depressa* M. Bieb.) *G. lydia* Boiss. (= *G. rumelica* Velen.)	*G. tinctoria* *G. ovata* *G. depressa* *G. lydia* *G. rumelica*
Spartioides Spach	*G. subcapitata* Pančić. *G. pilosa* L.	*G. subcapitata* *G. pilosa*
Voglera (P. Gaertn., B. Mey. & Scherb.) Spach	*G. carinalis* Griseb. *G. anatolica* Boiss.	*G. carinalis* *G. anatolica*
Asterospartium Spach	*G. sessilifolia* DC.	*G. sessilifolia*

Description of the cluster methods

Similarity distance based on the measure of disagreement was used in the cluster formation. When multiplied by 100%, this distance gives the degree of disagreement in percentage. Characteristically for this method, the closer taxa correspond to the lower correlation values and vice versa. This measure was chosen on the strength of the binary data coding by 0 and 1, reflecting the presence or absence of a given alkaloid in the species. Let us denote at random with x and y two species from Table 2, not necessarily different, and by x_i and y_i for $i=1,\ldots,21$ the values corresponding to the presence or absence of alkaloids characterising them. Then the similarity distance $d(x,y)$ between the species x and y shall be calculated by the relationship $d(x,y)=(number\ of\ x_i \neq y_i)/21$. The calculated similarity distances for the 10 investigated species are given in Table 2.

The linkage of the taxa into clusters of the 1st iteration during the cluster analysis was based on the selected similarity distance. In our case the greatest similarity corresponded to the minimum measure of disagreement. To link the clusters after the 1st iteration, various distances have been used, de-

Table 2. Measures of similarity based on the correlation of disagreement

	G. sessilifolia	*G. subcapitata*	*G. pilosa*	*G. carinalis*	*G. anatolica*	*G. lydia*	*G. rumelica*	*G. tinctoria*	*G. ovata*	*G. depressa*
G. sessilifolia	.00	.33	.19	.38	.33	.67	.14	.24	.24	.29
G. subcapitata	.33	.00	.24	.24	.19	.62	.29	.19	.19	.24
G. pilosa	.19	.24	.00	.19	.14	.76	.24	.14	.24	.29
G. carinalis	.38	.24	.19	.00	.05	.76	.43	.24	.33	.38
G. anatolica	.33	.19	.14	.05	.00	.71	.38	.19	.29	.33
G. lydia	.67	.62	.76	.76	.71	.00	.62	.71	.71	.57
G. rumelica	.14	.29	.24	.43	.38	.62	.00	.29	.19	.14
G. tinctoria	.24	.19	.14	.24	.19	.71	.29	.00	.10	.24
G. ovata	.24	.19	.24	.33	.29	.71	.19	.10	.00	.14
G. depressa	.29	.24	.29	.38	.33	.57	.14	.24	.14	.00

Table 3. Alkaloid composition of *Genista* species

Alkaloid type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
G. sessilifolia	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1
G. subcapitata	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0
G. pilosa	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	1	1	1	1
G. carinalis	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	1	1	0
G. anatolica	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	1	0
G. lydia	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
G. rumelica	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1
G. tinctoria	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0
G. ovata	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0
G. depressa	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0

Alkaloids: Sparteine (**1**); 11,12-Dehydrosparteine (**2**); 5-6-Dehydrosparteine (**3**); Retamine (**4**) 17-Oxosparteine (**5**); Lupanine (**6**); α-Isolupanine (**7**); 13-Hydroxylupanine (**8**); Nutaline (**9**); 4,13-Dihydroxylupanine (**10**); 13-Anegeloyloxilupanine (**11**); 4-Hydroxy-13 (2-methil, 2-buten)oxylupanine (**12**); 5,6-Dehydrolupanine (**13**); Anagyrine (**14**); Thermopsine (**15**); Baptifoline (**16**); Rhombifoline (**17**); N-Methylcytisine (**18**); N-Formylcytisine (**19**); Cytisine (**20**); Ammodendrine (**21**).

fined as functions of the similarity distances of the respective taxa included in hypothetical clusters that lead to different methods of linkage. Most frequently used were the methods of the (i) least distant neighbours; (ii) most distant neighbours; (iii) arithmetical or weighted mean arithmetical distances from the various pairs of distances; (iv) centres or weighted centres of weight of the distances from the various pairs in the clusters; and (v) Ward's method under which the distances between the clusters are defined so that the dispersion between two hypothetical clusters would maximally agree with Sneath & Sokal (1973).

The tree diagram presented in Fig. 1 is obtained on the basis of distances given in Table 2 and each of the methods (ii), (iii) and (v). The same tree diagram could be obtained when the ordinary correlation matrix, or the standard Euclidean distance, is used as a similarity distance, calculated for each two lines of data in Table 3 and interpreted as 21-measure vectors, and each of the methods of linkage (ii), (iii) and (v). The respective tree diagrams are not presented here, owing to the fact that the difference is only in the scale of linkage distances. Preference was given to the similarity distance based on the measure of disagreement, owing to the binary data coding and interpretation of the results.

The cluster analysis was carried out with the STATISTICA statistical programme package (Table 2).

Results

The aggregate cluster analysis of the 21 alkaloids identified in the 10 taxa has shown the following characteristic features (Fig. 1)

The species *G. carinalis* and *G. anatolica* showed the highest degree of similarity. These two species belong to one and the same section *Voglera*, which manifests a very good coordination between the chemical and morphological characteristics. Next closest to them is *G. pilosa*, with which they fall into one and same group, but the degree of similarity between them is lower. That group is considerably more isolated and differentiated from the other two taxa.

Very high similarity was observed between the species *G. tinctoria* and *G. ovata* referred to section *Genista*. *G. depresa* showed kindred alkaloid characteristics. The morphological characters of these taxa had given grounds to Gibbs (1968) to unify these three species into one taxon: *G tinctoria*. Our cluster analysis, however, did not confirm such a unification, but rather provided a prerequisite for a well defined species differentiation, irrespective of the similarity be-

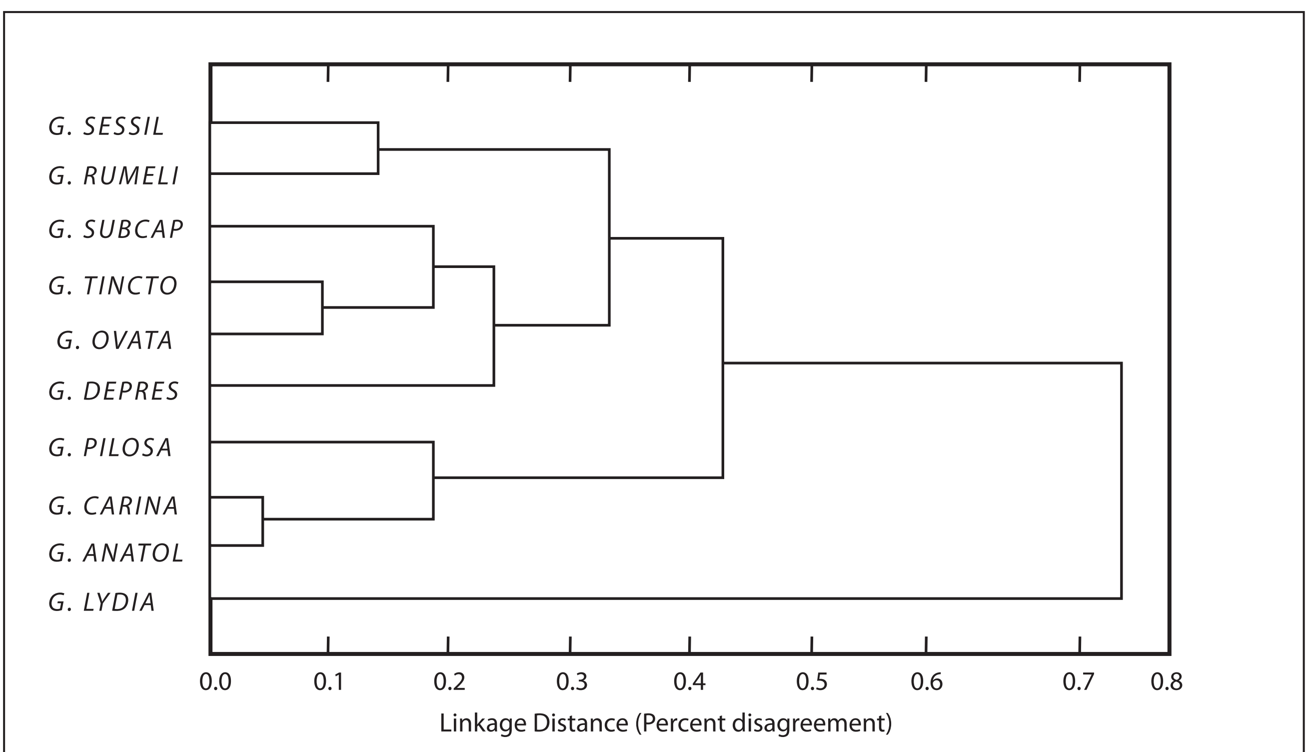

Fig. 1. Tree diagram of the alkaloid compounds in 10 species of genus *Genitsta*

tween them. Such a degree of similarity was observed between the above-mentioned species and *G. subcapitata* of section *Spartioides*.

The group *G. sessilifolia* – *G. rumelica* is particularly interesting from a taxonomic viewpoint. These two species also manifested a relatively high similarity in their alkaloid composition, although belonging to two morphologically comparatively distanced sections. Possibly, the evolution of the genus developed along two different lines: one from section *Genista* to section *Spartioides,* and the other from section *Genista* to section *Asterospartium.*

Particular interesting were the results obtained for the investigated population of *G. lydia* from the Eastern Balkan Range. This species belongs to the evolutionally lowest-level section *Genista*. It differentiates distinctly by its alkaloid composition from all other species of the genus. Alkaloids of the *Sparteine-Lupanine* type prevailed in it, characteristic for their formation in the earliest stages of the biogenesis. Presumably, such alkaloids biosynthesize mainly in the genera or species at the genocentre of the respective taxon. Alkaloid structure gets complicated with distancing from the primary genocentre towards the borderline parts of the taxon area (Satalino & Gottlieb 1980).

Limited distribution of this species – in the Balkan Peninsula and the western part of Asia Minor – confirms the assumption that it is in the early stage of its evolution. The low degree of oxidation of the alkaloids in this species (Christov & Evstatieva 2001) provides further evidence in support of its stage of development.

Discussion

The implemented cluster analysis of the alkaloids in the 10 investigated species of the genus brought forth certain taxonomic assessments for discussion. Thus in section *Genista* the species *G. tinctoria, G. ovata* and *G. depressa*, in spite of their great similarity, showed sufficient dissimilarities presuming their differentiation into separate species. In the same section the species *G. rumelica* distinctly differentiates from *G. lydia* by its alkaloid composition and they cannot be referred to one and the same species. These results support the taxonomic scheme accepted by Kuzmanov (1976).

In section *Spartioides* the two investigated species, *G. subcapitata* and *G pilosa,* were very different in their alkaloid composition. These differences were reaffirmed by their morphological characteristics too: in the first species the flowers were gathered into capitulae, in the second they were in elongated loose bunches. Probably this has something to do with different evolutionary trends in their development.

In section *Voglera* the two studied species, *G. carinalis* and *G. anatolica*, show high coordination and aggregation within the section.

The presence of triplex leaves in *G. sessilifolia* in section *Asterospartium* testifies to a more advanced evolutionary stage of that taxon. This is confirmed by the prevalence of alkaloids of the *Cytisine* and *Anagyrine* type, characteristic of the final stages of the biogenesis. Along with this, the presence of alkaloids of the *Sparteine-Lupanine* type characteristic of the initial stage of the biogenesis directly relates this species to the representatives of section *Genista.*

Conclusions

The cluster analysis of the alkaloid composition of 10 species of genus *Genista* growing spontaneously in Bulgaria has shown the highest degree of similarity between *G. carinalis* and *G. anatolica*, which supports their referring to section *Voglera*. The degree of similarity by their alkaloid composition of *G. tinctoria*, *G. ovata* and *G. depressa,* on the one hand, and *G. lydia* and *G. rumelica*, on the other, is sufficiently great and they should not be unified into one taxon (Gibbs 1968). This corresponds to the taxonomic scheme for differentiation of species according their morphological characters suggested by Kuzmanov (1976).

The cluster analysis of alkaloid composition outlines well the similarities of the sections *Genista* and *Voglera,* on the one hand, and of the section *Genista* with sections *Asterspartium* and *Spartioides,* on the other. The investigated population of *G. lydia* is characteristic with the presence of alkaloids with the earliest stage of biogenesis, which differentiates it distinctly from the other representatives of the genus.

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