TAXONOMICAL STUDIES ON SESELI ELATUM L. AND ALLIED SPECIES. - I. SYSTEMATIC IMPLICATIONS OF SOME MORPHO-ANATOMICAL CHARACTERS.

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Keywords: Seseli elatum agg.; Umbelliferae; morphology; vittae; secretory canals; adaptive ecological significance.

Abstract:
In the Friuli-Venezia Giulia Region (NE-Italy) three populations of Seseli elatum agg. occur, which have different altitude ranges and different ecology. It is generally difficult to distinguish these three taxa by classical taxonomical characters. This paper presents a first contribution to the solution of the problem. We have examined the classical morphological characters, plus microcharacters of the fruit surface (SEM), and the secretory structures (canals, ampullae, vittae). We propose some new discriminant characters. The morpho-anatomical variation seems to be correlated with a gradient of decreasing temperature and aridity, and increasing elevation. The anatomical variation corresponds with variation in the biochemical production. The numerical classification of all characters indicates a rather isolated position of the population which occurs in xerophytic plant associations at low altitude.

Introduction
This report on some populations of the Seseli elatum agg. from Friuli-Venezia Giulia Region (NE-Italy), apparently never studied until now, is part of a research project on the italian Umbelliferae as a possibile source of flavonoids and coumarins (Corsi & Pagni, 1983; Pagni & al., 1985; Coassini Lokar & al., 1986a,1986b).

According to Poldini (1975), the population of Seseli elatum agg. in the Karst Region of the North Adriatic Sea and in the South Eastern Alps could be subdivided into two taxa: Seseli elatum subsp. gouanii (Koch) P.W. Ball (Karst Region) and Seseli elatum subsp. austriacum (Beck.) P.W. Ball. The latter occupies the eastern Pre-Alps, up to the Dinaric Karst mountains, being a latitudinal and altitudinal vicariant of the former. This subdivision, however, is far from being satisfactory on the basis of the available evidence (Poldini, 1975).

Aim of this study is to detect discriminant characters for a better characterization of critical populations within Seseli elatum agg. in the studied areas, and to determine the environmental effects on microcharacters and secretory structures.

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The microcharacters of the fruit epicarp proved to have a taxonomic value at species level for diagnostic purposes (Cappelletti, 1979a), and have also a pharmacognostic interest because they are recognizable also when the drug is powdered, allowing the detection of adulterant species (Cappelletti, 1979a; 1979b; 1980). Great taxonomic value is attached to the secretory structures, particularly to the vittae (Heywood & Dakshini, 1971). Vittae and secretory canals are also interesting since they appear to be the site of synthesis and/or of storing of biologically active secondary products (Crowden & al., 1969; Hegnauer, 1971). A good knowledge of the structure secreting the biologically active substances, is indispensable for any phytochemical and pharmacological study.

MATERIALS AND METHODS

Materials

Plants and ripe achenes from wild specimens were collected in the following communities referable to three associations.
- *Salvio-Euphorbietum fragiferae* Lausi & Poldini 1962, colonizing the scree slopes of the coastal Karst Region. The community indicates xerophytic conditions (populations of *Seseli elatum* subsp. *gouanii* (Koch) P.W. Ball, that in the text will indicated with *SeG*);
- *Saturejo-Brometum condensati seslerietosum variae* Poldini & Chiapella 1986, a montane grassland association of the Pre-Alps, which indicates mesophytic conditions (populations of *Seseli elatum* agg. indicated with *Sel*);
- *Moehringio-Gymnocarpietum* Jenni-Lips 1930 em. Lippert 1966 *geranietosum macrorrhizi* Poldini & T. Wraber 1969, a pioneer association on gravelly, stable calcareous - dolomitic scree slopes of the submontane belt; the community indicates more mesophytic conditions (population of *Seseli elatum* agg. indicated with *Se2*).

The three grassland associations are listed according to a gradient of decreasing thermo-xerophytism and are distributed along an increasing elevation gradient (from the minimum altitude of 100 m to the maximum altitude of 700 m).

For comparison, also material of *Seseli elatum* subsp. *austriacum* (Beck) P.W. Ball, collected in Carinthia (Austria), was examined.

Dried specimens of the examined plants are deposited in *TSB* (Herbarium Tergestinum of Department of Biology, University of Trieste, Italy).

Methods

*Light microscopy*

The gross morphological features of the achenes were investigated by means of a dissection microscope. For the anatomical aspects transverse and longitudinal sections (20 µ in thickness) were stained by different techniques (DelafIELD’s haematoxylin, alkanna tincture, Sudan III, at the same time with glacial acetic acid, the latter to show the essential oils) (Faure, 1914; Jensen, 1962). Investigations on the structural details were carried out on isolated vittae following the Kapoor & Kaul (1966) method. At least ten achenes of each populations were examined.
Scanning electron microscopy

The ripe fruits were fixed and soaked in 6% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9) at 0°C for one week, following the procedure described by Trevisan & al. (1983). The samples were then gradually dehydrated in acetone of increasing concentration, dried by critical point method in a Balzers Union Critical Point Dryer and coated with gold in an Edwards S 150 A Sputter Coater. Observations on at least ten achenes for each populations were made under a Philips 500 SEM (accelerating voltage of 12 KV) at the Department of Biology of the Trieste University.

Data analysis

All of the measures regarding morphological, anatomical and histochemical characters have been reported in a matrix.

Such a matrix includes both continuous and discrete variables. The latter have been standardized according the following formula:

\[
\frac{X - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}}
\]

where \(X\) is the value to be transformed, \(X_{\text{min}}\) is the lowest value in the matrix, \(X_{\text{max}}\) is the highest value in the matrix, and they were measured on an ordinary scale. A similarity matrix has been obtained from the data matrix, by applying the coefficient of Wishard (Jaccard) (Wishard, 1975). On the basis of this matrix, a dendrogram of the taxa has been obtained, using Average Linkage Clustering (Anderberg, 1973). In order to select those characters that have the highest discriminant power among the populations, the data matrix was submitted to program NESTOFL (Feoli & al., 1982a; 1982b).

RESULTS

Anatomical observations - Light microscopy

The root

Several secretory canals with roundish sections are present in the cortical parenchyma, especially in the phloem of all the three populations. They are of considerable size and tend to become thicker in the outermost portion of the parenchyma. In SeG the central pith is absent. In Se1 and Se2 the pith is large, with a high number of secretory canals.

The stem

The cross section of the stem in specimens of all the three populations reveals a reduced cortical parenchyma, an abundant layer of xylem, a reduced pith and phloem. The phloem presents numerous, small, flattened secretory canals. Above each phloem bundle there is a mechanical tissue overlying a small, rounded secretory canal. The pith is abundant in all of the populations. In Se1 and Se2 there are several secretory canals, and sometimes also a large cavity full of secretion. In
SeG the secretory canals are less frequent and of smaller size, and the cavity is always absent.

The leaf

The cross sections of the leaf lamina always show a very large number of secretory canals, which are arranged all around the leaf margin, and have the same structure of those observed in the other vegetative parts, with no marked differences among the three populations.

The petiole

The petioles reveal several large secretory canals, which are arranged in double row under the upper and the lower epidermis, and completely surround the vascular bundles.

The colour of the secretory canals in SeG is slightly different from that of the other two populations: this may suggest a slightly different chemical composition of the secretion.

The fruit

The fruits are always oblong-ovoid, with flat commisural surfaces and five very prominent ribs. They are slightly bigger in SeG. The average sizes are: SeG 2.79 x 1.5 mm; Sel 2.19 x 1.15 mm; Se2: 2.10 x 1.10 mm. The stylopodium is always conical and the styles are strongly bent downwards. In SeG the surface is hairless, with scarce striations on the intercostal spaces; in Sel the fruits are a little pubescent with abundant striations; in Se2 the fruits are densely pubescent and the striations on the intercostal spaces very numerous.

In Sel and Se2 the seeds are surrounded by a single, one-layered tegument, composed by cells containing lipophilic matter in correspondance with the commisural face; the internal seed tegument is one-layered, thick and strongly yellow-coloured. This tegument is probably of cellulose, since it is not coloured by fat colouring substances. The abundant endosperms contain lipophilic matter (Fig. 1). The main characters of the secretory structures are reported in Tab. 1. The fruits

Fig. 1 — Sel-Se2: the larger secretory canals ( ); are bright red coloured; the smaller vitta ( ) is orange-red coloured (x350).

Fig. 2 — SeG = Sel = Se2: wall thickness in a vitta. In evidence the polygonal wall cells (x900).

Fig. 3 — SeG: high number of septa in the vittae. The septa are one-walled, transverse, thin, divericated at the extremity, mid-amber-coloured (x400).

Fig. 4 — Sel = Se2: the septa are larger, one-walled, transverse, oblique or vertical, thin, divericated at the extremity, dark-amber coloured (x400).

Fig. 5 — Sel = Se2: droplets of fatty substances ( ) in a vitta (x420).

Fig. 6 — SeG: vittae ( ) and secretory canal (arrow) ( ) in the rib. Inside, endosperm with lipophilic matter (x350).
have a considerable number of vittae that are rather variable as regards their position. At the level of each vallecula there are 2-6 vittae in SeG, 2-3 vittae in Sel and 1-3 vittae in Se2.

In SeG and Sel, at the level of each rib, at each side of the bundle, there is sometimes a smaller vitta with rounded lumen. In Se2 there is no vitta at rib level.

The wall cells of the vittae are polygonal, with moderately thickened wall (Fig. 2). The vittae are dimorphic: those in correspondence with the commissural face are large, the dorsal ones are thin. Their size varies among the three populations, both as far as length and width are concerned. The sizes are: 0.15 - 4.08 x 0.03 - 0.20 mm in SeG; 0.30 - 3.39 x 0.06 - 0.21 mm in Sel; 0.0228 - 3.18 x 0.06 - 0.20 mm in Se2.

The vittae of SeG have tranverse, thin, double septa (Fig. 3). In Sel and Se2 larger, oblique or vertical septa are sometimes present (Fig. 4). They are always divaricated at the extremity and most frequent on the dorsal vittae. The anasto-

Fig. 7 — SeG: stylpodium, style strongly bent downwards and crown of calicine teeth (x80).

Fig. 8 — Sel = Se2: the crown of calicine teeth is less evident and more close to the stylopodium; the stylpodium is more conic (x40).

Fig. 9 — SeG: achene surface in the intercostal spaces. Scarce elongated and elliptical striations (x600).

Fig. 10 — SeG: typical elliptical striations (x2.500).

Fig. 11 — Sel: achene surface in the intercostal spaces (x600).

Fig. 12 — Sel: radially converging striations with a protruding form (x2.500).

Fig. 13 — Se2: achene surface in the intercostal spaces (x600).

Fig. 14 — Se2: radially converging striations with point shaped center (x2.500).

Fig. 15 — Se2: typical conical structure in a round area (x2.500).

Fig. 16 — Seseli elatum subsp. austriacum: typical conical structure in a round area (x1.250).
moses between vittae are frequent and almost exclusively present in the dorsal vittae. The articulations of the vittae have different length and colour in the three populations; in Se1 and Se2 they have evident droplets of a fatty substance (Fig. 5).

A secretory canal is present above the bundles. This is smaller in SeG, and presents several interruptions. The wall of the secretory canals is less thick than the one of the vittae.

The fruits always present ampulla-like structures, both along the secretory canals and at their extremity towards the stylopodium. These structures are numerous and small in SeG, far larger and slightly more numerous in Se1 and Se2.

The coloration of the secretory canals (bright red) differs from the one of the vittae (orange red), which suggests that the two types of secretory organs produce different substances. (Fig. 6)

**Microcharacters of the fruit surface**

SeG clearly differs from Se2 for the presence of a crown of calicine teeth at more than 0.5 mm from the stylopodium. They are so much reduced to appear as a wavy, prominent crown (Fig. 7). This structure is much less evident in Se1 and Se2, where it is located closer to the stylopodium (Fig. 8).

Typical cuticle striations are present in the intercostal spaces (valleculae). SeG is characterized by scarce elongated and elliptical striations (Figg. 9, 10). In Se1 valleculae and cuticle striations are most abundant (Fig. 11). They radially converge and sometimes assume a protruding form (Fig. 12). In Se2 valleculae and striations are abundant too (Fig. 13), radially converging to a point shaped center (Fig. 14).

In Se2 the surface of the valleculae appears distinctly rugose owing to the presence of protruding cell strips, which delimit round areas. Within each round
area, typical conical structures occur (Fig. 15), consisting of a center on which the epicarp cell outlines are evident, which is surrounded by an area where cell outlines cannot be well distinguished and where the epicarp surface is sulcated by radially elongated ridges and furrows. Secretory cavities are present within the pericarp, under the dome-shaped structures. The presence of typical structures in connection with underlying secretory canals represents the most relevant feature of the fruits of the investigated entity. Such epicarp structures, easily recognizable even in the powdered drug, allow an easy botanical identification. The conic forms, which are evident also at low magnification, are located only on the intercostal spaces. All of the epicarp striations are probably of waxy composition. The papillar forms observed in Se2 are very frequent in *Seseli elatum* subsp. *austriacum*; the valleculae have numerous striations, thickly convergent in prominent and pointed structures (Fig. 16).

In spite of marked coarctation phenomena, the surface features of the fruits can be easily recognized both in the fresh fruits and in those gradually dehydrated.

**Quantitative data analysis**

All of the quantitative characters measured in the plants of the three populations have been arranged in a matrix, which has been submitted to numerical classification, in order to quantify the degree of morphological affinity among the populations. The results of classification are shown in the dendrogram of Fig. 17a, where *Sel* and *Se2* have a very high degree of similarity (0.774), whereas *SeG* has a very low degree of similarity with *Sel* and *Se2* (0.123). The graph of distances (Fig. 17b) confirms the results of classification: the distance between the entities are as follows: *SeG* and *Se2*: 1.0; *SeG* and *Sel*: 0.877; *Sel* and *Se2*: 0.226.

The results of NESTOFL allowed to select the most discriminant characters for the identification of the three entities. In order of decreasing significance (*X^2* values in brackets) they are: presence/absence of conical structures (95.4), striations in the valleculae (94.0), form of the stylopodium and of the calicine crown (93.1), maximum number of vittae/achene (92.1), presence/absence of central pith in the roots (88.1), presence/absence of ampullae (88.0), number of septa/vitta (87.4), number of abortive vittae (86.1). Some characters that normally are considered of great taxonomical relevance, do not show a high degree of significance; they are: hair cover of the achenes (73.0), length (19.1) and width (5.3) of the achenes, number of vittae/achene (17.6), and position of the sepals on the stylopodium (13.6).

**Conclusion**

The morphometrical analysis showed that some characters, that normally have great diagnostic value within the *Umbelliferae*, such as size of the achene, presence or absence of hairs, number of vittae/vallecula, in our case allow to distinguish *SeG* from *Sel* and *Se2*, but not *Sel* from *Se2*.

Some new characters proved to have high diagnostic value within the three entities under study. They are: form of stylopodium, form of the crown of calicine
Table 1 — The features of the secretory structures occurring in the achenes.

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<th></th>
<th>Seg</th>
<th>Sel</th>
<th>Se2</th>
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<tbody>
<tr>
<td><strong>Vittae:</strong></td>
<td>From 2 to 6 well developed</td>
<td>From 2 to 3 vittae on each</td>
<td>From 1 to 3 vittae on each</td>
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<tr>
<td>collocation</td>
<td>vittae on each intercostal</td>
<td>intercostal space.</td>
<td>intercostal space.</td>
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<td></td>
<td>space</td>
<td>Sometimes small vitta at the</td>
<td>0 vittae at the level of each</td>
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<td></td>
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<td>level of each rib.</td>
<td>rib.</td>
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<td></td>
<td>1 small vitta on each side of</td>
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<td></td>
<td>vascular bundle at the level</td>
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<td></td>
<td>of each rib.</td>
<td></td>
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<tr>
<td><strong>Secretory</strong></td>
<td>1 on each rib, external to</td>
<td>1 more large on each rib,</td>
<td>1 more large on each rib,</td>
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<td>canals:**</td>
<td>the vascular bundle.</td>
<td>external to the vascular</td>
<td>external to the vascular</td>
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<td>collocation</td>
<td></td>
<td>bundle.</td>
<td>bundle.</td>
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<td><strong>Vittae:</strong></td>
<td>After 1 h easily isolated</td>
<td>After 1 h easily isolated</td>
<td>After 1 h easily isolated</td>
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<td>ease of isolation</td>
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<tr>
<td>with KOH*</td>
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<td><strong>Vittae:</strong></td>
<td>maximum 37</td>
<td>maximum 21</td>
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<td>number</td>
<td>minimum 19</td>
<td>minimum 15</td>
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<td>mean 26</td>
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<td></td>
<td>maximum</td>
<td>maximum 3.39</td>
<td>maximum 3.18</td>
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<td>length in mm</td>
<td>4.08</td>
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<td>3.18</td>
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<td></td>
<td>minimum 0.15</td>
<td>minimum 0.30</td>
<td>minimum 0.28</td>
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<td></td>
<td>mean 1.97</td>
<td>mean 1.97</td>
<td>mean 1.86</td>
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<td>maximum 0.20</td>
<td>maximum 0.21</td>
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<td>breadth in mm</td>
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<td>mean 0.09</td>
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<td>dark amber</td>
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<td>colour after</td>
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<td>treating with KOH</td>
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<td><strong>Septa:</strong></td>
<td>number/vitta</td>
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<td></td>
<td>maximum 20</td>
<td>maximum 14</td>
<td>maximum 12</td>
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<td>minimum 1</td>
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<td>minimum 1</td>
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<td>mean 7</td>
<td>mean 7</td>
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<td></td>
<td>one-walled, transverse, thin,</td>
<td>one-walled, transverse, obli-</td>
<td>one-walled, transverse, obli-</td>
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<td>divaricated at the extremity.</td>
<td>que or vertical, larger, diva-</td>
<td>que or vertical, larger, diva-</td>
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<td>ricated at the extremity.</td>
<td>ricated at the extremity.</td>
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140
Septa: form dimorphic: large at the com-misural face, thin at the ribs.

Secretory canals: frequent interrup-tion

Secretory canals: shorter breadty

Secretory canals: numerous and small

Vittae: frequent, almost exclusively in the dorsal vittae.

* On the differences between vittae and secretory canals see Corsi & Pagni, 1983.

* * This character is related to the greater or lesser wall thickness of vittae cells; darker colour and easiness of isolation indicate a considerable degree of thickening.

teeth, maximum number of vittae, presence of central pith in the root, presence of ampullae, number of septa/vitta and number of aborted vittae. These characters are proposed as a further evidence for the discrimination between the two closely related populations.

The population of SeG presents numerous partially developed or abortive vittae; this suggest that this entity presents a situation of variability characteristic of taxa with more recent origin. The high frequency of abortive vittae raises the question of their functional role. It is possible that they represent an adaptation to drier habitats, strongly different from the typical mesophytic habitats of Seseli elatum aggregatum. The high number of septa in vittae of SeG further suggests that the septa present a different chemical pattern, with localization of different chemical components. In another study (Coassini Lokar & al., 1986a) we have observed a higher production of essential oil in the fruits of SeG; this accords well with the fact that this entity has the highest number of vittae (essential oil percentage in: SeG = 0.82%, Se1 = 0.55%, Se2 = 0.50%).

The different coloration of the SeG vittae may be attributed to a different qualitative and/or quantitative composition of essential oil. The essential oil of the SeG fruits is characterized by β - bisabolene, that of Se1 by α - pinene, γ - terpinene and p-cimene, and that of Se2 by β - cariophyllene and γ - cadinene (Coassini Lokar & al., 1986a).

The numerous secretory canals and the large ampullae of Se1 and Se2, judging by their different colour, seem to contain other types of secretion. This has been confirmed by the results of other researches (Coassini Lokar & al., 1986b), that showed a higher proportion of furocoumarinic derivatives in these entities (percentage in: Se1 = 1.64%, Se2 = 1.92%, SeG = 1.01%).

The three entities present therefore a gradual modification of morphoanatomi-
cal characters, of secretory structures, and of biochemical secretions in correlation with ecological and altitudinal variations of the corresponding habitats, reflecting a gradient of decreasing thermo-xerophytism.

Increasing aridity is correlated to a higher number and larger size of vittae, and consequently to a higher production of essential oil. The number and the size of secretory canals and of the ampullae, and hence the production of furocoumarins, tend to increase with decreasing aridity.

The results of numerical classification indicate a rather isolated position for SeG in respect to the other populations, which, on the contrary, appear to be closely related.

-Se1 and Se2, however, seem to be sufficiently distinct to merit recognition at subspecies or varietal level.

The characters of Se1 and Se2 resemble those of Seseli elatum subsp. austriacum (e.g., hair cover, elongated form of the stylopodium, calicine crown closest to the stylopodium, striations with pointed center, conical structures). Therefore, it seems that these two populations of the Pre-Alps are systematically closest to this transalpine taxon.

It will be interesting to extend the same researches also to Seseli elatum subsp. austriacum and to other taxa of the genus, in order to better clarify the taxonomy of this difficult group.
References


