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Leontodon hispidus L.s.l.: a preliminary report

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#### 2. Material and methods

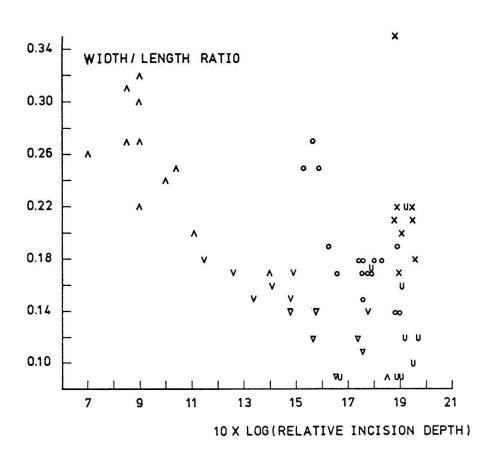
Populations from phytosociologically distinct vegetation units, sampled at various, mainly Swiss habitats were studied (Table 1). Part of the material was cultivated in a climatic chamber; the following conditions were applied: dry temperature at day 17°C, at night 10°C; day length 16 hrs.; light intensity 13000 Lux, relative air humidity 70 %. Methods used for morphological and cytological studies as well as those applied in experimental crosses are given in the respective chapters.

## 3. Morphology

## 3.1. Leaf shape

Leaf shape was described by means of the characters "width/length ratio", defined as the ratio of maximal leaf width to leaf length, "relative incision depth", defined as the ratio of isthmus width in the middle of the leaf, and "number of teeth", defined as one half of the total number of teeth per leaf. For each character, average values and standard deviations were determined from five leaves per individual.

For the assessment of the extent of phenotypical variation in leaf shape, samples from populations E and M were measured for the first time after a period of growth in a climatic chamber (see above) and, subsequently, after a period of growth in the garden. The characters "width/length ratio"



- grassland sample BOP (dry station)
- u grassland sample BOP
   (wet station: var.glabr.)
- A hyoseroides sample E
- v hyoseroides sample FAL (exposed station)
- hyoseroides sample FAL (less exposed station)
- x grassland sample M

Fig. 1. Leaf shape in wild samples

Table 1. Origin and code names of the investigated samples

| Taxon (var.*)          | Place of origin                      | Code<br>name | Habitat pH   |
|------------------------|--------------------------------------|--------------|--|
| glabratus              | Hombrechtikon(CH)<br>"Lutiker Riet"  | LUT          | wet hayland 7.4(+) (Molinietum)  |
|                        | Schönenberg(ZHCH) "Hinterbergriet"   | НВ           | wet hayland 6.3(-) (Molinietum)  |
|                        | Boppelsen (CH) "Boppelser Riet"      | BOP1         | wet/dry hayland 7.4(+) (Mesobrometum, Molinietum)                            |
| hyoseroides            | Zuerich (CH)<br>"Entlisberg"         | E            | open molasse scree 7.8(+) Sexposed slope,70%                                 |
|                        | Zuerich(CH)<br>"Fallätsche"          | FAL          | open molasse scree 7.7(+)<br>Sexposed slope 80-90%                           |
|                        | Wolfgang (CH)                        | WO           | open serpentine scree **   |
|                        | Davos (CH) "Totalphorn"              | TOT          | open serpentine scree 7.1(-) Sexposition                                     |
| "hysoreoides-<br>like" | Airolo (CH)<br>Albinasca             | AIR          | mesic pasture, 4.5(-) intensively grazed (Trisetum, Poo-Pruneletum)          |
|                        | Schilpario(I)<br>"Passo di Campelli" | CA           | mesic extensive 5.5(+/-) pasture (Nardetum, Seslerietum)                     |
| cf. dubius             | Davos (CH)<br>"Schiahorn"            | SCHI         | alpine meadow ** (on dolomite)   |
|                        | Davos (CH)<br>"Salezerhorn"          | SAL          | alpine meadow<br>(on silicate)   |
| hispidus               | Kirlovods (SU)                       | KIR          |  |
|                        | Pyatigorsk (SU)                      | PY           |  |
|                        | Zuerich-town(CH) "Kantonsschule"     | М            | lawn, cut frequently (+)   |
|                        | Zuerich-Hockler                      | HOE          | mesic meadow 6.5(+) (Lolio-Cynosuretum)                                      |
|                        | Stallikon(CH)                        | STAL         | dry/wet hayland 7.4(+) (Mesobrometum)  |
|                        | Boppelsen (CH)                       | BOP2         | dry-mesic hayland (Mesobrometum)   |
|                        | Küttingen (CH)                       | TTMB         | over 10 year old 8.0(+) dry fallow land (Tetragonolobus-Mesobrometum)        |
|                        | Küttingen(CH)                        | TCMB         | 6 years old,dry follow 7.8(+) land; S.Eexp.slope, 50% (Teucrio-Mesobrometum) |
|                        | Küttingen(CH)                        | ARRJ         | (mesic,manures hayland ** 2-3 cuttings per year (Arrhenatheretum)            |

<sup>\*</sup> according to HEGI(1929) (+) calcareous soil (-) not calcareous soil

<sup>\*\*</sup> not measured

Table 2. Phenotypical variation in leaf shape: mean values for three leaf parameters, mean standard deviations and mean variation in time

| Sample M<br>(8 individuals)                    | N of teeth | Width/length<br>ratio | Relative<br>incision depth |  |  |
|--|------------|-----------------------|----------------------------|--|--|
| climatic chamber                               | 9.2 + 1.5  | 0.21 ± 0.02           | 0.71 + 0.06                |  |  |
| garden   | 10.4 ± 0.9 | 0.21 ± 0.02           | 0.59 ± 0.06                |  |  |
| mean variation<br>in time<br>(absolute values) | 1.3        | 0.02                  | 0.12                       |  |  |
|  |            |                       |                            |  |  |
| Sample E (10 individuals)                      | N of teeth | Width/length<br>ratio | Relative<br>incision depth |  |  |
| climatic chamber                               | 8.0 ± 1.4  | 0.24 ± 0.04           | 0.11 ± 0.04                |  |  |
| garden   | 8.0 ± 1.3  | 0.22 ± 0.03           | 0.17 ± 0.04                |  |  |
| mean variation in time (absolute values)       | 1.4        | 0.03                  | 0.06                       |  |  |

and "number of teeth" were similar in samples E and M (table 2) so that variation ranges for both samples overlapped. On the other hand, the values for "relative incision depth" differed clearly and variation ranges were overlapping. The differences established at the  $10^{-6}$ -level of significance with the T-test for unpaired data. Relatively high average values and standard deviations of "relative incision depth" were observed in some E-individuals, which were infected by mildew.

The range of variation in time and the range of momentary intrapopulational variation were comparable for both samples. Apparently, phenotypical variation in leaf shape equals or prevails over genetically fixed variation within the samples.

Differences in number of teeth among various population found in samples kept under controlled conditions fall within the range of phenotypical variation (table 3). It should be mentioned that the leaf shape of some FAL plants from less exposed places was closely similar to that of glabratus plants (fig. 1). With a single exception, the ratio width/length was similar for all investigated samples; the deviating sample FAL

Table 3. Leaf shape in cultivated samples; mean values and standard deviations over five leaves per individual (figures for wild samples M, E and FAL are given for comparison).

| Sample                                   | N of teeth  | Width/lenght ratio  | Relative<br>incision depth  | n. of invest.<br>individuals     |  |  |  |  |  |  |  |
|--|---|---|---|----------------------------------|--|--|--|--|--|--|--|
| Samples kept in climatic chamber         |   |   |   |                                  |  |  |  |  |  |  |  |
| M<br>BOP<br>CA<br>SCHI<br>E<br>WO<br>PAR | 9.2 ± 1.5<br>8.2 ± 0.9<br>9.6 ± 1.3<br>9.7 ± 1.0<br>8.5 ± 1.5<br>9.0 ± 1.8<br>9.5 ± 1.6 | 0.21 ± 0.03<br>0.20 ± 0.03<br>0.25 ± 0.04<br>0.24 ± 0.08<br>0.25 ± 0.02<br>0.22 ± 0.03<br>0.27 ± 0.04 | 0.40 ± 0.11<br>0.24 ± 0.13<br>0.58 ± 0.07<br>0.09 ± 0.04<br>0.09 ± 0.02 | 8<br>6<br>10<br>7<br>9<br>5<br>5 |  |  |  |  |  |  |  |
|  | Wild samples  |   |   |                                  |  |  |  |  |  |  |  |
| M (27.10.75)                             | 7.1 ± 1.1   | 0.22 ± 0.06   | 0.82 ± 0.06   | 8                                |  |  |  |  |  |  |  |
| (27.10.75)<br>E<br>(26.10.75)            | 6.9 ± 0.9   | 0.26 ± 0.05   | 0.82 ± 0.06   | 11                               |  |  |  |  |  |  |  |
| FAL (4.11.75)                            | 7.8 ± 1.4   | 0.14 ± 0.03   | 0.36 ± 0.15   | 13                               |  |  |  |  |  |  |  |

of L. hyoseroides differed from all other samples, including the hyseroidessamples.

"Relative incision depth" was comparable in most of the hyseroides-samples (E, WO, TOT and PAR), but the sample FAL was aberrant again. Relative incision depth greatly contributes to the inter-populational differentiation of leaf shape (fig. 1); its apparent differentiation within the hyseroides-group suggests however that the character is of a minor taxonomical importance.

## 3.2. Leaf thickness

Leaf thickness of one leaf each per individual was measured by a transection in the middle of the leaf (table 4). The *hyseroides*-sample E apparently had thicker leaves than the grassland-sample M, when kept in the same environment, whereas hybrids between E and M appeared to be intermedi-

ate. Absolute values for both garden samples were similar. Average leaf thickness of samples E and M from the wild was smaller than that in the cultivated samples, but again differed significantly for both samples (table 7). However, broad overlappings occurred when both samples were kept under different conditions: potted individuals from both populations developed thicker leaves. This feature was so pronounced in the originally thin-leaved M-individuals that leaf thickness of these plants overlapped with the normal values for the *hyseroides*-plants.

Table 4. Leaf thickness in various cultures

|              | Mean values and standard deviations (in mm) |                        |                               |                        |                            |                        |  |  |  |  |  |
|--------------|---|------------------------|-------------------------------|------------------------|----------------------------|------------------------|--|--|--|--|--|
| Sample       | Garden at<br>the<br>Institute               | Number<br>of<br>plants | Garden at<br>Höngger-<br>berg | Number<br>of<br>plants | Pot<br>cultures            | Number<br>of<br>plants |  |  |  |  |  |
| E<br>ExM/MxE | 0.50 ± 0.08                                 | 10                     | 0.46 ± 0.04                   | . 4                    | 0.61 ± 0.07<br>0.53 ± 0.06 | 8<br>12                |  |  |  |  |  |
| М            | $0.30 \pm 0.06$                             | 8                      | 0.28 ± 0.06                   | 5                      | 0.44 ± 0.06                | 8                      |  |  |  |  |  |

underlined: overlapping of samples E and M.

## 3.3. Size of stomata

Samples from populations E and M were kept under identical conditions during one winter season in a climatic chamber (see methods) and subsequently transferred in open soil, remaining there during one summer season. Part of the experimentally obtained  $F_1$  -strains from both populations, including some hybrids, were potted and grown in open air. The length of the 5 larger, closed stomata at one leaf each per individual was measured (table 5). Size differences of stomatal cells between E- and M-individuals were significant under all conditions, significance levels ranging from  $10^{-2}$  to  $10^{-4}$  (T-test unpaired data). No significant change in stomatal size was observed in individuals kept in the climatic chamber and transferred to the garden. However, stomatal size of the potted samples from both populations was significantly higher than that of plants from the climatic chamber as well as these from the garden. Significance levels between the garden

and the potted plants were  $10^6$ - and  $10^{-3}$  for samples E and M, respectively. Because of absence of a strict genetical determination, stomatal length seems unsuitable as a diagnostic character. It indicates, however, different developmental responses of various populations of L. hispidus L. s. 1.

Table 5. Size of stomata

|              | Mean values and standard deviations (in mm) |                        |             |                        |                            |                        |  |  |  |  |  |
|--------------|---|------------------------|-------------|------------------------|----------------------------|------------------------|--|--|--|--|--|
| Sample       | Climatic<br>chamber                         | Number<br>of<br>plants | Garden      | Number<br>of<br>plants | Pot<br>cultures            | Number<br>of<br>plants |  |  |  |  |  |
| E<br>ExM/MxE | 0.042±0.002                                 | 10                     | 0.042±0.002 | 10                     | 0.047±0.002<br>0.045±0.003 | 8<br>12                |  |  |  |  |  |
| M            | 0.035±0.003                                 | 8                      | 0.037±0.001 | 8                      | 0.044±0.002                | 8                      |  |  |  |  |  |

## 3.4. Number of involucral leaves

The variation pattern seemed different in various samples (table 6). A low degree of individual variation was observed in sample E: in 14 individuals, 3 - 4 heads per individual were found and a deviation of more than 2 was noted only once. Total population variation appeared to be limited in populations E, M and BOP, whereas populations AIR and CA varied much more; in the latter populations a second modal value i.e. 21, apart from the normal one i.e. 13, occurred. A trend towards increase of the number of involucral leaves was also observed in the alpine sample SAL (table 7) and the Caucasic samples KIR and PY.

The number of involucral leaves might be related to the number of achenes per head: the average value of achene number amounted to 147 (14 heads) in CA-plants, whereas the average values for samples E, M and BOP were 58 (83 heads), 78 (93 heads) and 64 (58 heads), respectively. The intrapopulational variation in the number of achenes might be connected with the vitality of particular individuals.

Table 6. Variation in the number of involucral leaves.

|        |   |   |    |    | 1  | Numb | er | of | inv | volu | ıcra | al I | Leav | <i>r</i> es |    |    |    | 10-00-00-00-00-00-00-00-00-00-00-00-00-0 | Number      |
|--------|---|---|----|----|----|------|----|----|-----|------|------|------|------|-------------|----|----|----|--|-------------|
| Sample | 8 | 9 | 10 | 11 | 12 | 13   | 14 | 15 | 16  | 17   | 18   | 19   | 20   | 21          | 22 | 23 | 24 | of<br>plants                             | of<br>heads |
| E      | 2 | 1 | 3  | 2  | 3  | 67   | 4  | 1  |     | 1    |      |      |      |             |    |    |    | 46                                       | 84          |
| М      |   |   |    | 1  | 1  | 58   | 12 | 2  | 4   | 3    | 3    | 2    | 1    | 1           |    |    |    | 45                                       | 88          |
| BOP    |   |   | 1  |    | 1  | 30   |    | 1  | 1   |      |      |      |      |             |    |    |    | 35                                       | 35          |
| AIR    |   |   |    |    |    | 6    |    |    | 3   | 3    | 3    | 1    | 4    | 10          |    |    |    | 28                                       | 30          |
| CA     |   |   |    |    |    | 8    | 6  | 8  | 7   | 2    | 1    | 3    | 3    | 6           | 1  | 2  | 1  | 27                                       | 48          |
| KIR/PY |   |   |    |    |    |      | 1  |    |     |      | 1    |      | 1    | 6           |    |    | 1  | 7  | 10          |

## 3.5. Hairiness

#### 3.5.1. Hair morphology

Filiform hairs, which occur in all varieties of *L. hispidus* L. s. 1., invariably consisted of a single cell row with one or a few enlarged terminal cells. On the other hand, complex stellate hairs were subject to both individual and populational variation manifesting itself in a surprising number of forms (fig. 2).

Hair morphology in *L. hispidus* was sometimes found to show congruencies with other species: for instance, bending hair rays which normally occur in *L. incanus* Schrank and in *L. taraxacoides* Mérat (PITTONI 1974) were also observed in an alpine population WO of *L. hyoseroides* (fig. 2).

Number of rays per hair was liable to a remarkable individual variation (fig. 2). However, modal ray numbers, i. e. the most frequently occurring number of hair rays per leaf, appeared to be fairly constant in given individuals. The modal ray number characterized as well the populations and no variation was observed in the offspring obtained from experimental crosses between populations with the same modal ray number. In contrast, variation in modal ray number was observed between 4 hybrids of a cross between plants from the northern grassland population M and the southern hyseroides-like grassland-population CA. The modal ray number of hairs was accordingly considered in the present study as a reliable character in hair morphology and was used for the description of populations (table 7).

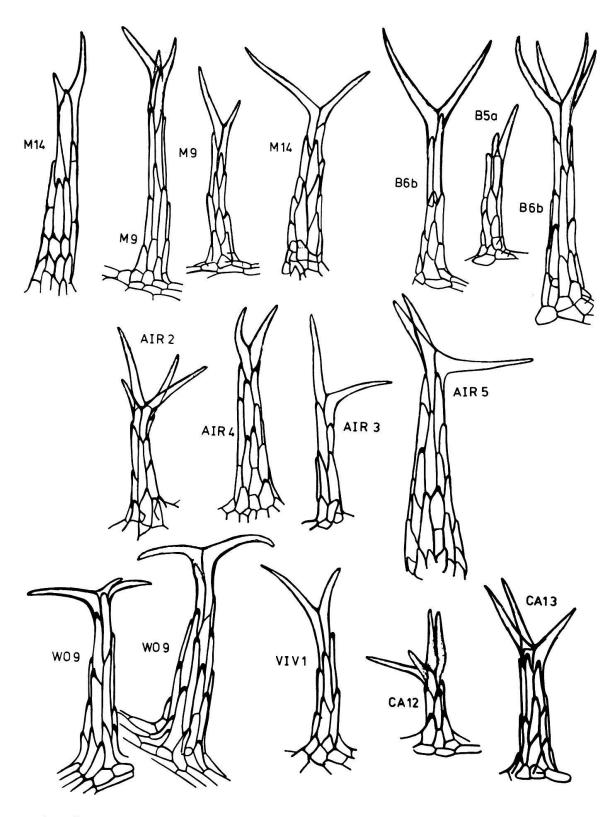


Fig. 2. Various forms of stellate hairs in Leontodon hispidus L. s. 1.

Table 7. Interpopulational differentiation of some morphological characters.

| Sample  | Modal value<br>of ray<br>number               | Hair<br>length(mm)   | Mean leaf<br>thickness                                       | Mean number<br>of involucral<br>leaves               |
|---|---|--|--|--|
| LUT (7) HB (3) STAL (2) BOP (5) M HOE ARRJ TTMB | 2<br>2<br>2<br>2<br>2<br>2<br>2<br>3 - 2<br>2 | 0.5 - 0.7<br>0.4 - 0.6<br>0.5 - 0.5<br>0.4 - 0.5<br>0.4 - 0.6<br>0.4 - 0.9<br>0.4 - 0.7<br>0.5 - 1.0 | 0.39<br>0.27<br>0.32<br>0.33<br>0.23<br>0.25<br>0.26<br>0.25 | 13.4<br>12.8<br>14.3<br>12.9<br>13.1<br>13.0<br>13.7 |
| TCMB AIR SAL SCHI FAL TOT(2)                    | 2 - 3<br>3<br>3<br>3 - 2<br>-<br>2            | 0.4 - 0.8<br>0.3 - 0.6<br>0.4 - 1.0<br>0.4 - 0.7<br>-<br>0.6 - 0.6                                   | 0.29<br>0.26 !<br>0.36<br>0.39<br>0.40<br>0.46               | 12.6<br>17.4<br>16.0<br>13.1<br>12.1                 |

Note: Numbers of haired individuals in populations with less than 50 % haired individuals are given in brackets. Samples ARRJ, TTMB, TCMB consisted of 10 individuals, all other samples consisted of 20 individuals.

The following values of modal ray number were observed:

 $\mbox{MRN}$  = 1 occurring solely in experimentally obtained hybrids (CA x M and E x M).

MRN = 2, mainly found in the grassland populations from the Swiss Plateau; it occurred as well in some *hyseroides*-populations TOT and WO, where a few individuals were sparsely haired.

MRN = 3 was found in the grassland population TTMB, TCMB and ARRJ (the Jura of Aargau), AIR (central Alps) and also in the Alpine population SCHI and SAL (table 7).

The obtained results suggest that the "modal ray number" might be geographically differentiated. It should be mentioned, however, that similar values occur in otherwise physiognomical distinct populations, inhabiting distant areas: the investigated Jura populations on the one hand and the Alpine populations on the other hand, all have a congruent range of modal values with a highest value of 3. Though it may serve as an indication of

regionally occurring gene exchange, the modal number of rays seems to be of rather limited taxonomic value.

# 3.5.2. Length of hairs

Average hair length was measured in the middle leaf part of each two leaves per individual (table 7). All investigated populations, both with a strongly varying leaf shape as well as those with relatively constant leaf shape, showed a wide range of hair length. Consequently, hair length seems to be of a limited adaptive significance. It should be noted that a very dense and remarkable short, indument (0.3 mm) was typical for some individuals in population CA; such induments seem far less variable than less dense and longer haired induments.

### 3.5.3. Density of hairs

The populations of *L. hyseroides* from Entlisberg and Fallätsche were both completely glabrous. On the other hand, some sparsely haired individuals, probably resulting from a gene exchange, occurred in the samples TOT and WO. Other investigated populations either consisted of both hairless as well as haired individuals or were completely haired (PY, KIR). Some haired individuals were found in the *glabratus*-populations LUT and HB and in the populations STAL and BOP. On the other hand, the populations M, HOE, AIR, SCHI, SAL and all three Jura populations (TTMB, TCMB and ARRJ) consisted of more than 50 % of haired individuals.

In 3 out of 4 sparsely haired  $F_1$ -individuals from a hybridization between a hyoseroides- and a grassland-parent a temporal variation in presence/absence of leaf hairs was observed: formerly haired individuals turned glabrous. Such kind of variation was further noticed in 2 sparsely haired individuals from the heterogeneous population BOP.

Leaf hairs were partly very coarse; coarser hairs occurred in the median part of the involucral leaves, and most frequently had fewer rays. The indument of the marginal part of the involucral leaves was less dense than that of the leaves. Hairs were irregularly distributed over the involucral leaves. Their presence/absence was controlled at 4 inflorescences

per individual pf each 4 F<sub>1</sub>-individuals from 8 crosses (viz. 2 crosses E x E, 2 crosses CA x CA, 2 crosses M x M and 2 crosses E x M: all 8 individuals from reciprocal hyseroides-crosses E x E were hairless; all 8 individuals from reciprocal intrapopulational grassland-crosses M x M were constant in the presence of hairs; their leaves had a dense indument whereas those of their parents were intermediate to densely haired. The hairiness apparently is a character with expression varying in different parts of an individual, all 8 plants from crosses CA x CA showed a constant absence of involucral hairs whereas their leaves sometimes were sparsely haired (parents totally glabrous!). All 8 individuals obtained from interpopulational crosses E x M invariably had glabrous leaves; rosette leaves were also glabrous except for a single sparsely haired individual.

#### 3.5. The seed

The following seed characters were studied: seed length, number of ribs, number of long setae and colour of the seed coat. Seed length without pappus was measured in samples M and E. Individual variation between different fruit heads was investigated in 10 seeds per head for 5 plants from the former sample, whereas the latter one was studied in 13 individuals. Variation between various fruit heads most frequently proved to be nearly the same as that within a single head. Individual variation within either population was well-marked, the respective average values being 5.9 mm ± 0.5 mm for sample E (17 individuals) and 5.7 mm ± 0.8 mm for sample M (16 individuals). It should be noted that variation within the population M is partly due to a local polyploid differentiation: the longest seeds in sample M were found in autotriploids, whereas the sample E consisted solely of diploids. The ranges of variation in seed length overlapped for both samples.

The number of seed ribs was counted in 24 heads from 11 E-individuals and in 34 heads from 13 M-individuals (1 seed per head). The number of ribs was always 5; in some individuals ribs were not clearly visible.

The variation ranges of the number of long pappus setae overlapped largely in samples E and M. Lowest values were found in sample M (11), highest values in sample E (18), whereas interpopulational hybrids were intermediate.

Seed coats were mostly darker in E- than in M-plants. Transversal ribbing of the coat sometimes seemed deeper in E- than in M-individuals.

### 4. Cytology

#### 4.1. Somatic chromosome numbers

Somatic chromosome numbers of *L. hispidus* L. s. 1. were previously reported from various parts of Europe (table 8). The incidental occurrence of triploids seems to constitute the main variation in chromosome number. Triploid plants were found in larger samples; the finding of triploids might therefore be related to sample size.

The present counts were performed on root tips of germinating seeds and potted plants. Fresh root tips were collected at noon and pretreated over 1.5 hrs in 0.05 per cent aqueous colchicine at room temperature (± 20°C). They were subsequently transferred to 3: 1 acetic alcohol and, after overnight fixation, kept in lacto-propionic orceine until further processing. The root tips were gently cooked for 2 - 2.5 min. and squashed in a fresh drop of the lacto-propionic orceine. Squashes were made permanent by removing the cover slide in butyl alcohol; then object slide and cover slide were processed in xylol and embedded in caedax. Loss of material could be avoided by covering the cover slide with a thin layer of albumen-glycerine and heating it over a flame.

The chromosome numbers found in the course of the present study are in agreement with previous data (table 8): most of the studied plants represented the diploid level (2n=14, fig. 3) and only in a single sample some autotriploids were found (2n=21,fig.4). In addition, some aneuploids (2n=16, figs 5 - 6) were found in the offspring of the triploids.