

# Cytology

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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*

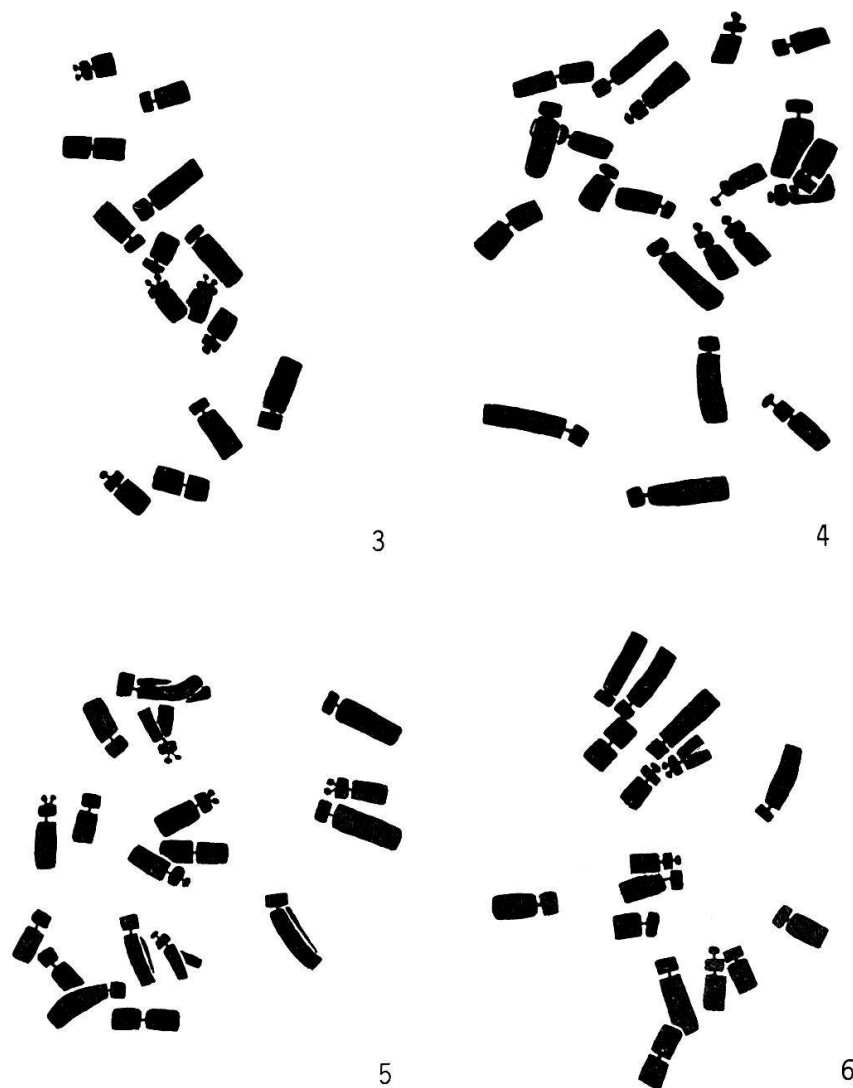
Somatic chromosome numbers of *L. hispidus* L. s. l. were previously reported from various parts of Europe (table 8). The incidental occurrence of triploids seems to constitute the main variation in chromosome number. Triploid plants were found in larger samples; the finding of triploids might therefore 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 ( $\pm 20^{\circ}\text{C}$ ). 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 ( $2n=16$ , 18, figs 5 - 6) were found in the offspring of the triploids.

Table 8. Somatic chromosome numbers in *L. hispidus* L. s. l.

Place of origin	2n	Author(s)	Remarks
Great Britain	14	ELLIOT 1950	Deviating karyotype.
	14	FINCH 1967	58 plants in samples from 8 counties;
	21		1 sample (14 plants) with triploid.
	14	ROUSI 1973	
	14	EDMONDS et al. 1974	
Netherlands	14	GADELLA and KLIPHUIS 1968	
France	14	GUINOCHET and LOGEOIS 1962	Deviating karyotype in material from the Maritime Alps.
	14	GADELLA and KLIPHUIS 1970	
Portugal	14	ROUSI 1973	var. <i>glabratus</i> (Koch) Bischoff
	14	FERNANDES and QUEIROS 1971	
GERMANY	14	TISCHLER 1934	
	14	ROHWEDER 1937	
	14	FINCH 1967	
Switzerland	14	FINCH 1967	9 individuals
	14	present study	<i>L. hispidus</i> L.s.str.: 1 sample(39 plants) with 2 triploids; 2 samples(12 and 5 plants) without triploids.
	14		<i>L. hyoseroides</i> Welwitsch: 3 samples(5, 6 and 15 plants) without triploids.
Austria	14	FINCH 1967	
	14	NILSSON and LARSEN 1971	
	14	PITTONI 1974	var. <i>glabratus</i> (Koch) Bischoff and var. <i>hispidus</i>
Poland	14	SKALINSKA et al. 1964	var. <i>glabratus</i> (Koch) Bischoff from the High Tatra with deviating karyotype.
USSR	14	ROUSI 1973	<i>L. danubialis</i> Jacq.
Czechoslovakia	14	FINCH 1967	
	14	ROUSI 1973	
Italy	14	PITTONI 1974	var. <i>hispidus</i> , var. <i>hyoseroides</i> (Welw.) Beck and var. <i>pseudocrispus</i> Sch.-Bip.
Yugoslavia	14	PITTONI 1974	var. <i>glabratus</i> (Koch) Bischoff
Rumania	14	ROUSI 1973	
Bulgaria	14	KUZMANOV and GEORGIEVA 1976	
Sweden	14	BERGMAN 1935	Deviating karyotype.
	14	STEBBINS et al. 1953	
Finland	14	FINCH 1967	
	14	PITTONI 1974	



Figs. 3 - 6. *Leontodon hispidus* s. l.: root-tip metaphases.

3. A normal diploid ( $2n=14$ ). 4. An autotriploid ( $2n=21$ ).

5, 6. Aneuploid plants ( $2n=18$ ,  $2n=16$ ). (c.) 1500 x.

#### 4.2. Chromosome morphology

For the study of chromosome morphology, drawings of 8 metaphases per individual at comparable stages of contraction were made with the aid of a camera lucida at 2975 x magnification. The choice of metaphases was based on the long arm length of the metacentric chromosomes, the criterion of choice ranging within 0.5 mm on drawing paper (about 0.17  $\mu$ m).

Chromosome complements of *L. hispidus* L. were previously described by BERGMAN (1935), ELLIOT (1950), GUINOCHE and LOGEOIS (1962), SKALINSKA et al. (1964), FINCH (1967), FERNANDES and QUEIROS (1971) and ROUSI (1973). The descriptions of BERGMAN (1935) as well as SKALINSKA et al. (1964) remain isolated. ELLIOT (1950) as well as GUINOCHE and LOGEOIS (1962) mentioned two pairs of metacentric chromosomes, whereas only one metacentric or sub-metacentric pair was reported in the works of FINCH (1967), FERNANDES and QUEIROS (1971) and ROUSI (1973). Satellites restricted to the subacrocentric pairs were reported in all later studies.

The best document study is that of FINCH (1967); apart from the metacentric pair "C" he distinguished for the first time two longer subacrocentric pairs named "A" and "B", and for shorter ones, viz. "D", "E", "F" and "G", satellites being confined to the latter group. FINCH mentioned a variation in satellite visibility and size. His observations were later confirmed by ROUSI (1973).

In the present study, the karyotypes of four plants from the grassland-population M are compared with those of five plants from the *hyoseroides*-population E (table 9, fig. 7). The differences between both samples are small in relation to the interindividual variation. The general idiograms (fig. 7) are in agreement with the results of FINCH. The two pairs of longer subacrocentric chromosomes (A and B), on the one hand, and the three shortest pairs of the four SAT-pairs (E, F and G), on the other hand, appeared to be undistinguishable from each other. FINCH's values, as derived from his diagram, don't considerably exceed the range of interindividual variation in samples E and M. In the present material satellites were not always visible, variation occurring within single root tips. However, satel-

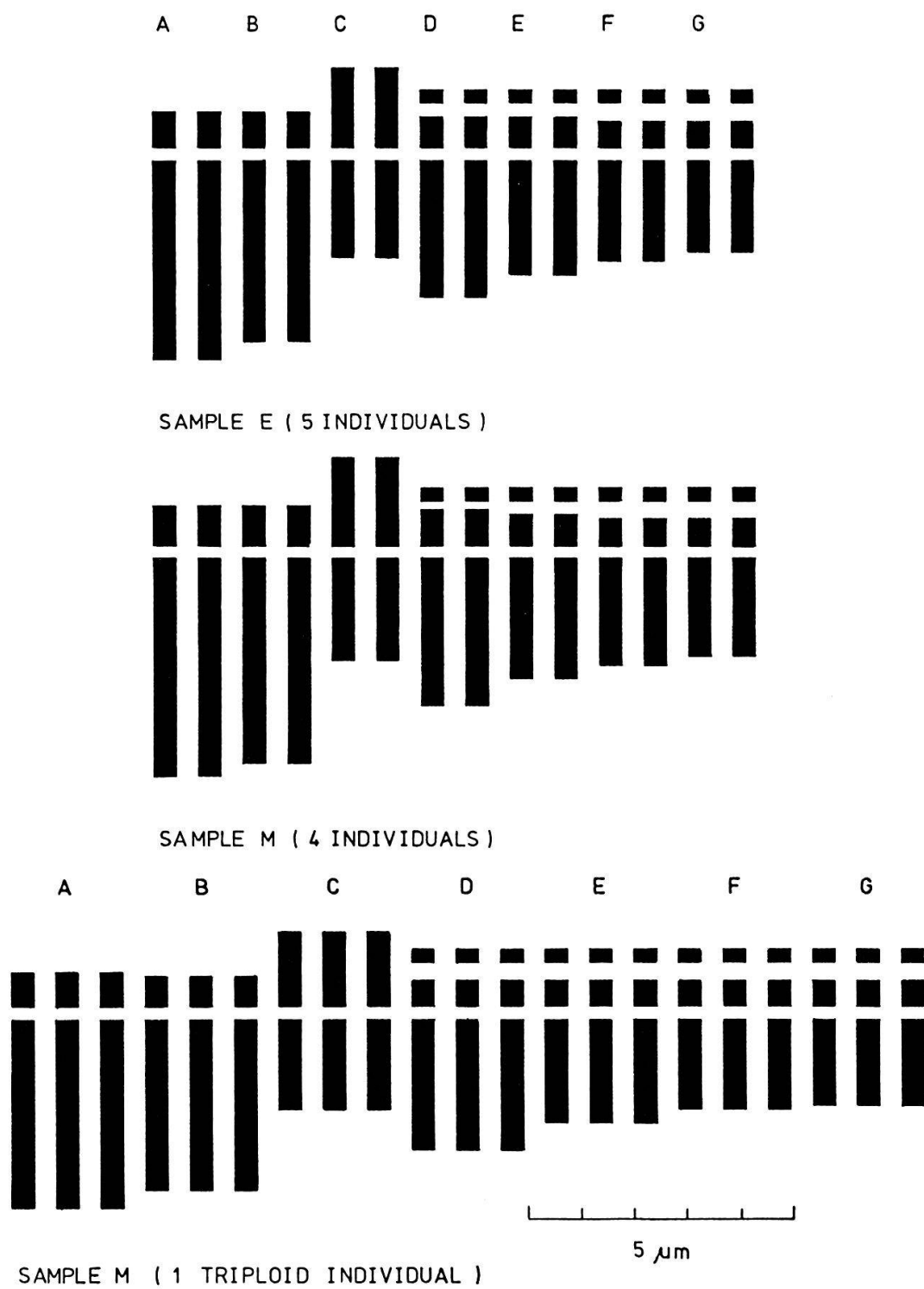


Fig. 7. Chromosome complements in *Leontodon hispidus* s. l.

Table 9. Chromosome morphology

Mean arm length (sa = short arms; la = long arms) and standard deviations in units of 0.336 $\mu$ m			
Chromosome pair		Complement E (5 individuals)	Complement M (4 individuals)
A	s.a.	2.05 $\pm$ 0.23	2.27 $\pm$ 0.32
	l.a.	11.14 $\pm$ 0.44	12.27 $\pm$ 0.49
B	s.a.	1.97 $\pm$ 0.20	2.15 $\pm$ 0.32
	l.a.	10.37 $\pm$ 0.42	11.43 $\pm$ 0.46
C	s.a.	4.60 $\pm$ 0.33	5.00 $\pm$ 0.36
	l.a.	5.38 $\pm$ 0.32	5.87 $\pm$ 0.41
D	s.a.	1.84 $\pm$ 0.21	1.94 $\pm$ 0.24
	l.a.	7.73 $\pm$ 0.51	8.34 $\pm$ 0.45
E	s.a.	1.73 $\pm$ 0.22	1.70 $\pm$ 0.24
	l.a.	6.42 $\pm$ 0.36	6.78 $\pm$ 0.43
F	s.a.	1.58 $\pm$ 0.22	1.62 $\pm$ 0.20
	l.a.	5.73 $\pm$ 0.30	5.96 $\pm$ 0.28
G	s.a.	1.60 $\pm$ 0.20	1.60 $\pm$ 0.18
	l.a.	5.18 $\pm$ 0.30	5.44 $\pm$ 0.28

Relative arm length (length arm/total karyotype length)					Ratio short arm/long arm		
Chromosome pair		E	M	Data of FINCH (1967) *	E	M	Data of FINCH (1967) *
A	s.a.	1.5	1.6	1.6	0.18	0.19	0.21
	l.a.	8.3	8.5	7.9			
B	s.a.	1.5	1.5	1.6	0.19	0.19	0.21
	l.a.	7.7	7.9	7.8			
C	s.a.	3.4	3.5	3.5	0.86	0.85	0.83
	l.a.	4.0	4.1	4.2			
D	s.a.	1.4	1.4	1.5	0.24	0.23	0.26
	l.a.	5.8	5.8	5.9			
E	s.a.	1.3	1.2	1.3	0.27	0.25	0.29
	l.a.	4.8	4.7	4.5			
F	s.a.	1.2	1.1	1.2	0.28	0.27	0.28
	l.a.	4.3	4.1	4.1			
G	s.a.	1.2	1.1	1.0	0.31	0.29	0.25
	l.a.	3.9	3.8	3.8			

\* FINCH's data are derived from his general idiogram

lite frequencies were similar for both investigated samples (table 10). In agreement with FINCH's observation, satellites were confined to the four shorter subacrocentric pairs (D, E, F and G). They seem to be more frequent in the shorter pairs. The varying visibility of satellites might be attributed to the preparation technique.

The variation range of short arms of the SAT-chromosomes was about as large as their own size, viz. 1.5 - 2.0 mm on the drawings, corresponding with 0.50 - 0.67  $\mu$ m. This variation was remarkably related to the variation in satellite visibility (satellited arms are generally shorter). In table 9 the average short arm length of SAT-chromosomes is based only on satellited short arms. The variation in longer arms might be attributed to interindividual differences in the metaphase contraction stage.

Arm ratios proved to be rather unreliable for homologue identification because of the small dimensions of the short arms. The two pairs of longer subacrocentric chromosomes (A and B) as well as the four pairs of shorter subacrocentric SAT-chromosomes (D, E, F and G) accordingly appeared to be indistinguishable. The increase of the ratio: short/long arm from the longer to the shorter SAT-pairs was related to the progressive length decrease of the long arm. Since the variation ranges of long arm length were overlapping, the three shortest SAT-pairs might be identical in morphology. In contrast, the long arm length distribution of the longest SAT-pair suggest its distinct morphology.

None of the data obtained in the present study give some indications for discontinuous variation in chromosome morphology within or between samples E and M. A superficial check on populations M, BOP, TOT and SCHI by means of the single metaphase drawings from 12, 12, 11 and 5 individuals respectively, did not reveal any remarkable deviation either: a general chromosome morphology in the *hyoseroides* variety might be identical with that of the grassland varieties. Deviating earlier descriptions might represent rare exceptions without ecological significance.



Table 10. Percentage occurrence of satellites

Chromosome	Sample E	Sample M
D	44	35
E	50	49
F	69	70
G	70	69

## 5. Reproductive behaviour

### 5.1. *Seed setting*

Plants from populations M, B0, CA, E, WO and PAR were submitted to selfings and crosses in order to gain insight into their breeding behaviour and to check on the possible sterility barriers. Five plants each from the populations E and M were left in the garden for an open pollination.

Forced crosses and both free as well as forced selfings were carried out in the greenhouse and climatic chamber. Selfed flowers were isolated with aseptic gauze bags. Cross-populations were performed by brushing reciprocally two flower heads each 24 hrs at noon over the whole or practically the whole period (up to maximal 5 days) of flowering. The flower heads were subsequently isolated. A practical problem with some influence on the seed setting percentage was raised by the fact that two flower heads, which one decided to cross, did not always open and wither on the same day. As a result, the achenes in the centre of the delayed flower head were sometimes not pollinized by the partner flower head giving rise to a centered spot of empty seeds.

The flowering period in the garden ranged from two up to eight days, varying between three and six days for most of the plants. The shorter flowering periods coincided with sunny weather, whereas cool, cloudy or rainy days coincided with the longer periods. Most flower heads with a seed set-