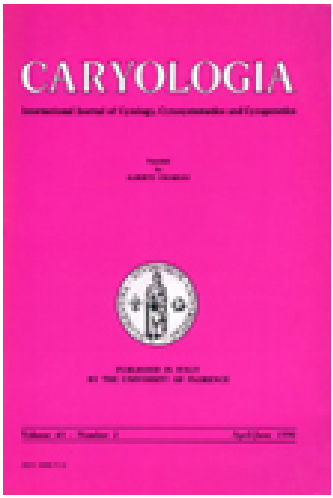

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DNA Content, Karyotype Structure Analysis and Karyotype Symmetry in Ranunculus L. (Ranunculaceae). Italian Species Belonging to Sections Flammula (Webb) Benson and Micranthus (Ovcz.) Nyarady

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DNA CONTENT, KARYOTYPE STRUCTURE ANALYSIS AND KARYOTYPE SYMMETRY IN *RANUNCULUS* L. (RANUNCULACEAE). ITALIAN SPECIES BELONGING TO SECTIONS *FLAMMULA* (WEBB) BENSON AND *MICRANTHUS* (OVCZ.) NYARADY

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SUMMARY — DNA microdensitometric measurements and karyotype analysis show that in spite of variable ploidy level (2*x* - 16*x*) and mean chromosome size (0.37 - 0.83 pg), genomic structure is remarkably constant throughout both the examined Sections. Karyotype symmetry, defined numerically for each species, has been compared with that of other species of *Ranunculus* (Subgen. *Ranunculus*). Such comparison confirms that both the examined Sections include relatively advanced species as suggested by DAVIS (1960) on the grounds of macromorphic and ecological elements.

INTRODUCTION

The genus *Ranunculus* (incl. *Oxygraphis*, *Batrachium*, *Ficaria*, *Ceratocephalus*, *Krapfia*, *Halerpestes* etc.) comprises about 400 species (ENGLER 1964) distributed mostly in the temperate and cold regions of the boreal hemisphere but also in the tropics or the austral zone. TUTIN (1964) reports 122 *Ranunculus* species in Europe and PIGNATTI (1982) 85 species in Italy. Both Sections, FLAMMULA and MICRANTHUS, include paludal species with ovate and lanceolate leaves and slender roots. The species belonging to Section FLAMMULA have peduncled flowers and short beaked achenes while the species of Section MICRANTHUS have smaller, sessile, or nearly so, flowers and beaks long at least as half of the achene. Not all the Floras assign infrageneric rank recognition to such differences as done by OVCZINNIKOV (1937), NYARADY (1953) and TUTIN (1964). MAIRE (1964) and COOK *et al.* (1986) include *R. lateriflorus* among the species of Section FLAMMULA.

DAVIS (1960) considers his informal LANCIFOLII group (equivalent to Section FLAMMULA) as a «rather specialized group». Indeed D'OVIDIO *et al.* (1985) comparing the karyotype structure of more than 30 species of *Ranuncu-*

lus have found that *R. flammula* and *R. ophioglossifolius* showed the most asymmetric complements. Degree of asymmetry and degree of specialization are thought to be positively correlated in many plant groups: «trends toward greater asymmetry are more common than their reversal» during the evolution of higher plants (STEBBINS 1970).

We have now extended the analysis of karyotype structure to all the species of Sections FLAMMULA and MICRANTHUS growing in Italy (six and one species respectively). Our principal aim is to compare these data with corresponding karyological data available (GOEPFERT 1974; D'OVIDIO *et al.* 1985) for other species of *Ranunculus*. Thus this paper is meant as a further contribution to the definition of karyotype variation and evolution in the genus *Ranunculus*.

MATERIALS AND METHODS

Origin of the examined plants and vouchers.

Sect. MICRANTHUS		
R. lateriflorus DC.	1) Lazio, edges of the lake of Rascino (Rieti).	RO, 20.06.86 (leg. D'Ovidio)
Sect. FLAMMULA		
R. ophioglossifolius Vill.	1) Sardegna, near the main road from Pattada (Sassari) to Bultei (Sassari), in a stream.	RO, 16.05.86 (leg. D'Ovidio, Marchi, Masci et Millozza)
	2) Sardegna, Campeda district, Road N131 near the cross-road for Bolotana (Nuoro).	RO, 11.05.86 (leg. D'Ovidio)
	3) Lazio, Castel Porziano (Roma), «Piscinale Grande».	RO, 11.05.86 (leg. Cipriani)
R. revelieri Boreau	1) Sardegna, near the main road from Pattada (Sassari) to Bultei (Sassari), in a stream.	RO, 30.05.86 (leg. D'Ovidio, Marchi, Masci et Millozza)
	2) Sardegna, Campeda district, Road N131 near the cross-road for Bolotana (Nuoro), edges of a stream.	RO, 16.05.86 (leg.D'Ovidio)
R. flammula L.	1) A. Adige, Renon Plateau (Bolzano), *Pinus sylvestris* wood glade, in a ditch.	RO, 19.06.86. (leg. Marchi)
	2) Lazio, Rascino lake (Rieti), south-west border.	RO, 25.06.86 (leg. D'Ovidio, Marchi et Masci)
R. reptans L.	1) Piemonte, Oleggio (Novara) right bank of the Ticino river.	RO, 21.07.86 (leg. D'Ovidio et Soldano)
R. fontanus Presl.	1) Calabria, Sila Piccola district, in a boggy plain named Ciricilla (Catanzaro).	RO, 25.05.86 (leg. Capineri, Marchi et Masci)
R. lingua L.	1) Toscana, lake of Chiusi (Siena), near the western shore.	RO, 11.07.85 (leg. D'Ovidio, Marchi, Masci et Millozza)

Nuclear DNA measurement. - Relative nuclear DNA contents were determined by Feulgen microdensitometry using a Barr & Stround type GN5 integrating microdensitometer. Root tip nuclei were prepared following essentially the procedure described by Mc LEISH and SUNDERLAND (1961). *R. repens* 4C nuclei, whose DNA absolute content of 23.08 pg has been calculated by SMITH and BENNETT (1975), have been used as standard. For each species 30 nuclei were measured in each of three replicates.

Definition of karyotype asymmetry. - GREILHUBER and SPETA (1976) define separately each component of karyotype asymmetry by means of an index: REC (index of REsemblance among Chromosomes) that accounts for chromosome size variation within the complement and the SYi (SYmmetry index) that represents average centromere position.

REC is the mean of n-1 *rec* values; $rec = (t_i:t_1) \times 100$; t stands for chromosome total length, t_1 for longest chromosome length, i for each of the other chromosomes (2, 3,... n). SYi is the ratio between the mean value of short arms and the mean value of long arms multiplied by 100.

REC index equals 100 if all the chromosomes of the complement share the same total length. REC index approaches zero if the ratio between the length of each of the shorter chromosomes and the length of the longest approaches zero.

SYi index equals 100 when all the chromosomes of the complement have arms of equal length. SYi index approaches zero when the short arm mean length approaches zero.

Karyotype definition. - Somatic metaphase plates were obtained from actively dividing root tips. After 2-3 h pretreatment in a 0.04% colchicine water solution, root tips were fixed from 30 min to 18 h in a 3:1 absolute ethanol-glacial acetic acid mixture, hydrolised in 1N HCl solution at 60°C for 7 min and stained for 90 min in leucobasic fuchsin. Coloured ends were separated from the rest of the root tip, reduced to a cell suspension in 45% acetic acid and squashed.

The best plates have been photographed and printed at 2000×. Chromosomes have been measured and measures have been worked out by means of MCGURK and RIVLING's (1983) program (slightly modified) for the Apple IIe computer implemented with a digitizer.

Thus there are four ideal karyotypes delimiting all the possible ones:

1. When REC = 100 and SYi = 100, both symmetry components are at their maximum, the karyotype is composed by median chromosomes of equal size.

2. When REC = 0 and SYi = 0 then asymmetry is at its maximum, the karyotype is made up by telocentrics where only one is commensurable while the rest is too small to be measured.

3. When REC = 100 and SYi = 0 then the karyotype is formed by telocentrics of equal size.

4. When REC = 0 and SYi = 100 then the karyotype is composed by metacentrics where only one is commensurable while the rest is too small to be measured.

Species code:

acr	*R. acris*	fla	*R. flammula*	par	*R. parviflorus*
ads	*R. adscendens*	fon	*R. fontanus*	pla	*R. platanifolius*
adu	*R. aduncus*	gar	*R. garganicus*	pol	*R. pollinensis*
alp	*R. alpestris*	gra	*R. gramineus*	rep	*R. repens*
ape	*R. apenninus*	ill	*R. illyricus*	rpt	*R. reptans*
arv	*R. arvensis*	lan	*R. lanuginosus*	rev	*R. revelieri*
aur4	*R. auricomus* s.l. 4×	lat	*R. lateriflorus*	sar	*R. sardous*
aur5	*R. auricomus* s.l. 5×	lin	*R. lingua*	sce	*R. sceleratus*
aur6	*R. auricomus* s.l. 6×	mil	*R. millefoliatus*	ser	*R. serbicus*
aur7	*R. auricomus* s.l. 7×	mon	*R. monspeliacus*	thm	*R. thomasii*
bre	*R. brevifolius*	mnt	*R. montanus* s.s.	tho	*R. thora*
bru	*R. brutius*	mur	*R. muricatus*	vel	*R. velutinus*
bul	*R. bulbosus*	oph	*R. ophioglossifolius*	ven	*R. venetus*
bll	*R. bullatus*	ore	*R. oreophilus*		
cal	*R. calthifolius*	pal	*R. paludosus*		

OBSERVATIONS

In Table 1 microdensitometric nuclear 2C DNA values in picograms (column 6) and linear karyotype values in micrometers (column 11) are reported for each species. Dividing both such sets of values by the ploidy level coefficient of each species, mean genomic DNA content (column 7) and mean basic set length (column 12) were derived. In Fig. 1 values of columns 6 (left ordinate) and 7 (right ordinate) have been plotted. The total nuclear DNA content of polyploid nuclei equals the expected multiple of diploid *R. lateriflorus*. Diploid *R. ophioglossifolius* is decidedly out of line, since its nuclear content is twice the content of the other diploid *R. lateriflorus* and equal to tetraploid species values. Such a situation is much less evident from karyotype length comparison (Fig. 2) but karyometry allows only rough quantitative DNA evaluations. However karyometry is still the only way to define karyotype structure and degree of symmetry. Fig. 3 represents the haploid complement of seven species drawn to scale using the «genomic DNA contents in *R. lateriflorus* units» listed in Table 1 column 8.

GREILHUBER and SPETA (1976) symmetry indices have been calculated for each species (column 13 and 14) and used to plot a scatter chart (Fig. 4 area 10). In Fig. 4 also the karyotype symmetry position for 36 other *Ranunculus* species, or biotypes, as defined by D'OVIDIO *et al.* (1985) are represented (lines delimit infrageneric groupings).

Finally the index of REsemblance among Chromosomes and the SYmmetry index for each of fortythree *Ranunculus* have been tabulated (Tab. 2) and plotted (Fig. 4) in order to define the position of Sections FLAMMULA and MICRANTHUS species within genus *Ranunculus*.

The two GREILHUBER and SPETA indices are inversely correlated to a high degree of significance ($P < 0.001$).

TABLE 1

			Microdensitometry (2C DNA amounts in pg)						Karyometry					
					This paper									
	Ploidy level	Life-cycle type	(GOEPFERT 1974)	(SMITH and BENNETT 1975)	Number of nuclei measured	Nuclear content	Mean genomic content	Genomic DNA content in *R. lateriflorus* units	Plants	Nuclei	*2n* karyotype	Basic set	SYi	REC
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sect. Micranthus														
R. lateriflorus	2*x*	a	5.4	3.94	80	5.98 ± 0.29	2.99	1.00	3	6	40.51 ± 6.63	20.26	55.56	62.7
Sect. Flammula														
R. ophioglossifolius	2*x*	a	13.31		120	13.35 ± 0.23	6.68	2.23	3	8	68.54 ± 11.24	34.27	48.34	63.5
R. revelieri	4*x*	a			80	13.93 ± 0.55	3.48	1.16	2	5	121.43 ± 14.77	30.36	52.07	58.1
R. flammula	4*x*	p	14.46	13.07	120	14.36 ± 0.27	3.59	1.20	4	7	123.60 ± 22.69	30.90	54.08	60.9
R. reptans	4*x*	p			120	14.51 ± 0.48	3.63	1.21	3	5	106.01 ± 9.77	26.50	54.65	60.3
R. fontanus	6*x*	a			120	20.32 ± 0.61	3.39	1.13	3	6	138.42 ± 19.37	23.07	54.35	48.4
R. lingua	16*x*	p	49.58	51.6	120	51.86 ± 0.65	3.24	1.08	4	4	334.29 ± 17.31	20.89	50.87	61

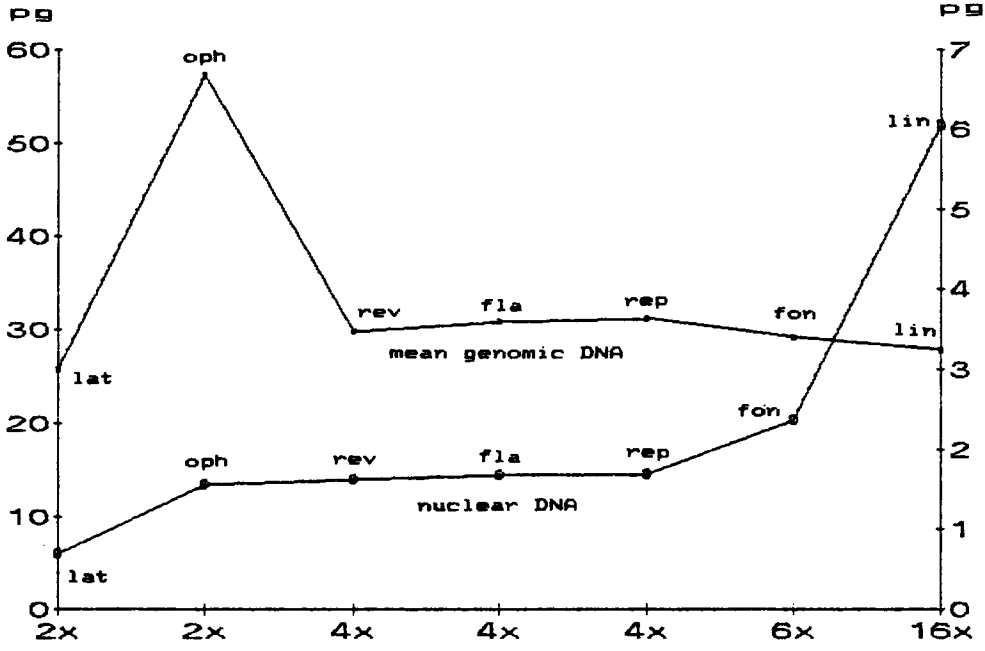

Fig. 1. — Whole nucleus (under) and genomic (above) DNA content for the seven species of *Ranunculus* of Sections *Flammula* and *Micranthus* growing in Italy. Species code: lat *R. lateriflorus*, oph *R. ophioglossifolius*, rev *R. revelieri*, fla *R. flammula*, rpt *R. reptans*, fon *R. fontanus*, lin *R. lingua*.

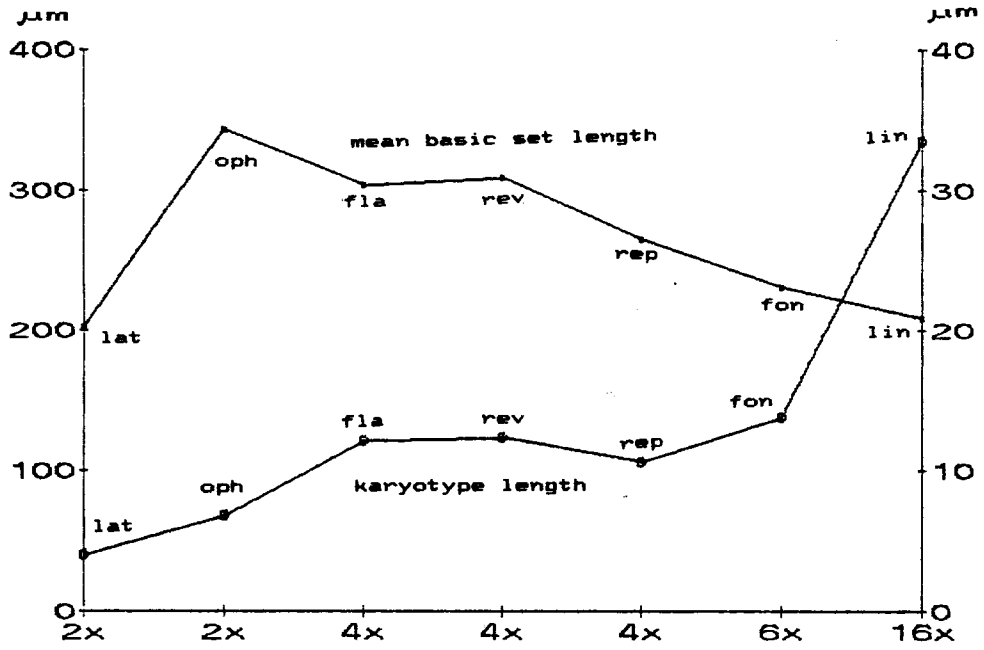

Fig. 2. — Whole aploid karyotype length (under) and mean basic set length (above) for the seven species of *Ranunculus* of Section *Flammula* and *Micranthus* growing in Italy. Species code: lat *R. lateriflorus*, oph *R. ophioglossifolius*, rev *R. revelieri*, fla *R. flammula*, rpt *R. reptans*, fon *R. fontanus*, lin *R. lingua*.

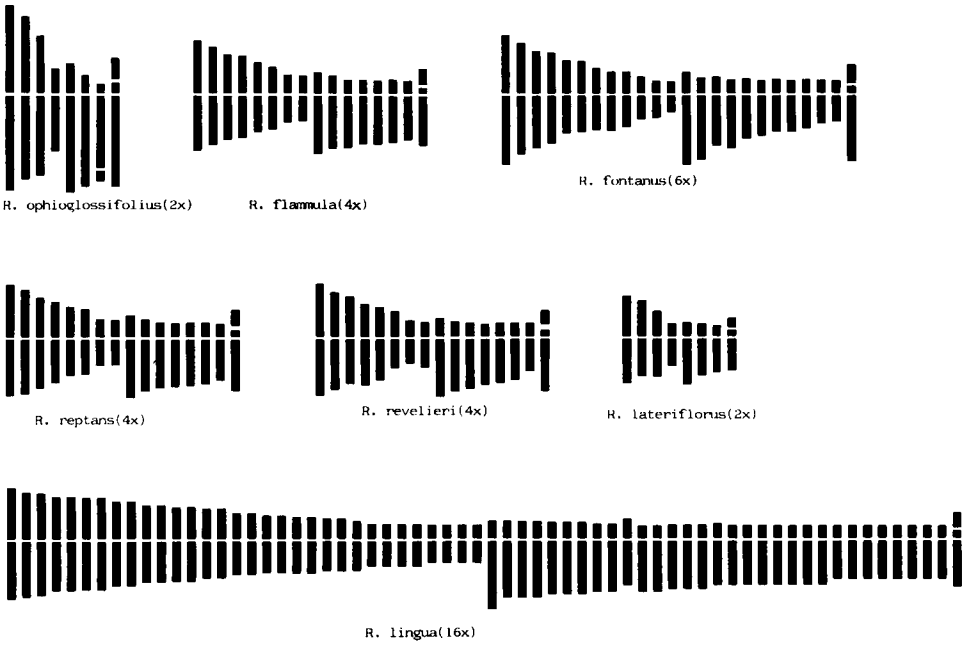

Fig. 3. — Representation of the aploid complements (idiograms) of the seven species of *Ranunculus* Sections *Flammula* and *Micranthus* growing in Italy drawn to scale their genomic DNA contents.

DISCUSSION

Nuclear DNA content. — The two diploid ($2n = 16$) species differ in nuclear DNA content in a 1:2 two-fold manner. The diploid showing the lower content (*R. lateriflorus*) appears as more likely involved in the origin of polyploids sharing with these the genomic DNA content. Diploids with the smaller chromosomes are supposed to be «pre-adapted» to polyploidy (DARLINGTON 1965).

Differences in nuclear DNA content have been correlated with many widely different morphological and functional characters (BENNET 1985). For instance GRIME and MOWFORTH (1982) suggest that, at least in some cases these differences can be explained as adaptation to different environmental conditions. GRIME and MOWFORTH have analysed nuclear DNA content and annual growth cycles in 162 species of the British flora. In a sample of 24 species they have found that the higher is the nuclear DNA content the earlier is the time of shoot expansion. This is possible because there is a temperature range low enough to prevent cell proliferation but not low enough to prevent

growth by cell expansion. Plants with large nuclei, having also larger cells, can grow faster within such range than plants with smaller cells (provided the number of cells is about the same). GRIME and MOWFORTH also noticed that in their collection, plants with large nuclei and early shoot expansion are in a high proportion geophytes and grasses with geographical ranges centered on the

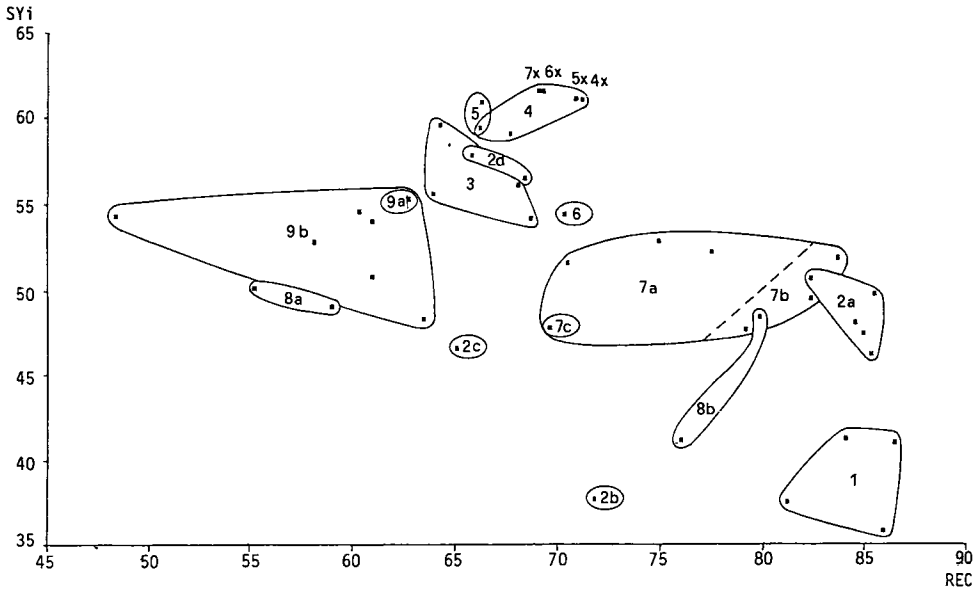

Fig. 4. — Scatter chart of symmetry indices in *Ranunculus* (Subgen. *Ranunculus*). The karyotype of each species is represented by a point whose coordinates are its REC and SYi symmetry indices. Numbered areas include species belonging to the same subgeneric taxon, either formal or informal. (Data partially from D'OVIDIO *et al.* 1985).

1. *Praemorsi* Davis (1960): *R. bulbosus* L. (2*x*), *R. adscendens* Brot. (2*x*), *R. repens* L. (4*x*), *R. thomasii* Ten. (2*x*). 2. *Rhizomatosi* Davis (1960): 2a *R. apenninus* Chiov. (2*x*), *R. pollinensis* (Terr.) Chiov. (4*x*), *R. montanus* L. s.s. (4*x*), *R. venetus* Huter (4*x*), *R. oreophilus* Bieb. (2*x*); 2b *R. aduncus* G. et G. (2*x*); 2c *R. brutius* Ten. (2*x*); 2d Sect. *Thora* DC. - *R. thora* L. (2*x*), *R. brevifolius* Ten. (2*x*). 3. *Heptabasici* (Rhizomatosi or Praemorsi species having $x=7$, instead of $x=8$, as basic chromosome number): *R. acris* L. (2*x*), *R. velutinus* Ten. (2*x*), *R. lanuginosus* L. (4*x*), *R. serbicus* Vis. (4*x*). 4. Subsect. *Epirotes* Prantl (1888): *R. auricomus* L. s.l. (4*x*, 5*x*, 6*x*, 7*x*), *R. sceleratus* L. (4*x*), *R. alpestris* L. (2*x*). 5. Sect. *Alpestres* (Prantl) Rapaics in Kert (1901): *R. platanifolius* L. (2*x*), *R. alpestris* L. (2*x*). 6. Sect. *Ranucella* (Spach) Freyn: *R. gramineus* L. 7. *Grumosi* Davis (1960): 7a Sect. *Xiphocoma* Ovcz. (1937) - *R. bullatus* L. (2*x*), *R. illyricus* L. (2*x*), *R. monspeliacus* L. (4*x*); 7b Sect. *Pterocarpa* Ovcz. (1937) - *R. paludosus* Poir. (4*x*), *R. millefoliatus* Vahl (2*x*), *R. garganicus* Ten. (2*x*); 7c Sect. *Ficaria* (Huds.) A. Gray (1895) - *R. calthifolius* (Rchb.) Bluff, Nees, Schauer (2*x*). 8. *Annui* Davis (1960) p.p., Sect. *Echinella* DC. (1824), Subsect. *Eubuthyranthus* Ser. *Arvenses* Prantl (1888): 8a *R. parviflorus* L. ($2n=4x=28$), *R. arvensis* L. (4*x*); 8b *R. sardous* Crantz (2*x*), *R. muricatus* L. (6*x*). 9. *Lancifolii* Davis (1960): 9a Sect. *Micranthus* (Ovcz.) Nyarady: *R. lateriflorus* DC.; 9b Sect. *Flammula* (Webb) L. Benson (1936) - *R. flammula* L. (4*x*), *R. fontanus* Presl (6*x*), *R. lingua* (16*x*), *R. ophioglossifolius* Vill. (2*x*), *R. reptans* L. (4*x*), *R. revelieri* Boreau (4*x*).

TABLE 2 - *Symmetry indices and their sum. Subgeneric taxa are numbered as in Fig. 4 and ordered according to increasing mean asymmetry.*

Subgeneric Taxon		Species	Code	Chromosome Number and Ploidy level	SYi	REC	SYi + REC
Epirotes	4, 5	*R. alpestris*	alp	$2n = 2x = 16$	59.45	66.15	125.60
	4	*R. sceleratus*	sce	$2n = 4x = 32$	59.09	67.62	126.71
	4	*R. auricomus s.l.*	aur7	$z = 7x = 56$	61.63	69.09	130.72
	4	*R. auricomus s.l.*	aur6	$2n = 6x = 48$	61.59	69.30	130.89
	4	*R. auricomus s.l.*	aur4	$2n = 4x = 32$	61.15	70.85	132.00
	4	*R. auricomus s.l.*	aur5	$z = 5x = 40$	61.12	71.15	132.27
Grumosi	7a	*R. monspeliacus*	mon	$2n = 4x = 32$	51.65	70.43	122.08
	7a	*R. bullatus*	bll	$2n = 2x = 16$	52.94	74.89	127.83
	7a	*R. illyricus*	ill	$2n = 2x = 16$	52.31	77.51	129.82
	7b	*R. garganicus*	gar	$2n = 2x = 16$	47.73	79.14	126.87
	7b	*R. millefoliatus*	mil	$2n = 2x = 16$	49.56	82.38	131.94
	7b	*R. paludosus*	pal	$2n = 4x = 32$	51.94	83.69	135.63
	7c	*R. calthifolius*	cal	$2n = 2x = 16$	47.80	69.58	117.38
Alpestres	5	*R. platanifolius*	pla	$2n = 2x = 16$	60.92	66.21	127.13
Rhizomatosi	2a	*R. oreophilus*	ore	$2n = 2x = 16$	46.20	85.32	131.52
	2a	*R. venetus*	ven	$2n = 4x = 32$	47.46	84.94	132.40
	2a	*R. montanus s.s.*	mnt	$2n = 4x = 32$	48.12	84.54	132.66
	2a	*R. apenninus*	ape	$2n = 2x = 16$	50.75	82.36	133.11
	2a	*R. pollinensis*	pol	$2n = 4x = 32$	49.83	85.51	135.34
	2b	*R. aduncus*	adu	$2n = 2x = 16$	37.71	71.76	109.47
	2c	*R. brutius*	bru	$2n = 2x = 16$	46.59	65.07	111.66
	2d	*R. brevifolius*	bre	$2n = 2x = 16$	57.89	65.79	123.68
	2d	*R. thora*	tho	$2n = 2x = 16$	56.58	68.36	124.94
Ranucella	6	*R. gramineus*	gra	$2n = 2x = 16$	54.45	70.30	124.75
Praemorsi	1	*R. repens*	rep	$2n = 4x = 32$	37.54	81.15	118.69
	1	*R. bulbosus*	bul	$2n = 2x = 16$	35.82	86.00	121.82
	1	*R. thomasii*	thm	$2n = 2x = 16$	41.26	84.10	125.36
	1	*R. adscendens*	ads	$2n = 2x = 16$	41.03	86.50	127.53
Heptabasici	3	*R. velutinus*	vel	$2n = 2x = 14$	55.64	63.93	119.57
	3	*R. serbicus*	ser	$2n = 4x = 28$	54.25	68.65	122.90
	3	*R. acris*	acr	$2n = 2x = 14$	59.59	64.21	123.80
	3	*R. lanuginosus*	lan	$2n = 4x = 28$	56.19	68.04	124.23
Annui	8a	*R. parviflorus*	par	$2n = 4x = 28$	50.16	55.17	105.33
	8a	*R. arvensis*	arv	$2n = 4x = 32$	49.08	58.93	108.01
	8b	*R. muricatus*	mur	$2n = 6x = 48$	41.20	76.04	117.24
	8b	*R. sardous*	sar	$2n = 2x = 16$	48.43	79.85	128.28
Lancifolii	9a	*R. lateriflorus*	lat	$2n = 2x = 16$	55.56	62.70	118.26
	9b	*R. fontanus*	fon	$2n = 6x = 48$	54.35	48.37	102.72
	9b	*R. revelieri*	rev	$2n = 4x = 32$	52.87	58.11	110.98
	9b	*R. ophioglossifolius*	oph	$2n = 2x = 16$	48.34	63.45	111.79
	9b	*R. lingua*	lin	$2n = 16x = 128$	50.87	60.95	111.82
	9b	*R. reptans*	rpt	$2n = 4x = 32$	54.65	60.30	114.95
	9b	*R. flammula*	fla	$2n = 4x = 32$	54.08	60.91	114.99

mediterranean region. In a mediterranean climate, where most of the annual rainfall occurs in winter and early spring, plants with big cells could be favoured because through the year they can vegetate actively for longer periods than plants with small cells.

Moreover DNA values differences through out the species examined here, whether determined or not by polyploidy, are aproximately integer multiples of the minimal value of 6 pg circa (*R. lateriflorus*). ROTHFELDS *et al.* (1966) observed that in *Anemone* and related genera (Ranunculaceae) DNA nuclear contents do not form a continuous series but appear to fit an arithmetic progression. More recently this phenomenon has been thoroughly studied in *Lathyrus* (NARAJAN 1982) and in *Nicotiana* (NARAJAN 1987). It has been confirmed also in *Clarkia*, *Allium* etc. (review in NARAJAN 1982). Also in these genera nuclear DNA distribution among species is discontinuous. Species can be clustered in separate DNA groups and members of each group have closely similar DNA amounts. DNA intervals between successive groups are very similar within the same genus and may vary among genera. The average interval is 3.71 in *Lathyrus*, 2.1 in *Clarkia*, 4.2 in *Allium*, 2.0 in *Nicotiana*. Moreover in genera where specific variation is associated with disploidy or polyploidy (e.g. *Allium* or *Nicotiana*) discontinuous changes show no correlation with numerical chromosome changes or with levels of polyploidy.

Is still not clear whether the shift of DNA content between one level and the other is attained by some kind of a saltatory event or by a continuous accretion of DNA. VIDA and MAJOR (1987) observed a 21% increase of genomic DNA content in synthetic autotetraploids of *Cristella hispidula* (Decne) Holttum (Filicopsida). Thus the induction of tetraploidy has determined a 242% increase of nuclear DNA; of such increase 83% can be ascribed to the ploidy level shift, but 17% to an abrupt variation in chromosome mean DNA content attained from one sporophytic generation to the next one. However VIDA and MAJOR (1987) warn that, on account of the peculiarities of the experiment, the observed differences not due to polyploidization could may not be the result of an amplification bound to become permanent, but also of rearrangement, heterochromatinization, chromatin diffusion or to the fact that nuclei were measured in cells undergoing differentiation.

Karyotype structure. — In the examined species of Sections MICRANTHUS and FLAMMULA, each chromosome of the basic set seems to hold fairly well its relative length and its centromere position troughout specific differentiations, variations of chromosome absolute size and number (Fig. 3).

An analogous situation, exhaustively documented by NARAJAN (1982), can be observed in *Lathyrus*. 21 diploid *Lathyrus* species represent a four-fold variation in their nuclear DNA amounts. In four of these species, presenting a two-fold variation in nuclear DNA amounts, chromosome DNA content was independently measured by spot microdensitometry. It appeared that from one

species to the other DNA changes were approximately of the same magnitude in all the chromosomes.

Karyotype symmetry. — Karyotype asymmetry is the resultant of two tendencies. One grows «through the accumulation of differences in relative size between the chromosomes of the complement» the other «through the shift of centromere position from median to subterminal or terminal» (STEBBINS 1971). STEBBINS (l.c.) suggests a measuring system based on separate evaluation of the tendencies. Such system classifies karyotypes in 12 categories.

Chromosomes having an arm ratio exceeding 2 are grouped in four frequency classes (none, 10-50%, 51-99%, all). These classes are plotted against three other classes obtained considering the ratio between the longest and the shortest chromosomes of the complement (ratios between 1 and 2, between 2 and 4, exceeding 4).

The purpose of this simplified classification is mainly to «look for associations between increasing asymmetry and other characteristic». Indeed morphological or ecological characteristics «are most easily scored in terms of alternative states rather than quantitatively».

However if the main purpose is to compare karyotype asymmetry with taxonomic groupings, there is no need to arrange karyotype asymmetry into categories. In other words if the purpose is to test the predictive value of taxonomic treatments by means of karyotype asymmetry (D'OVIDIO *et al.* 1985), the requirement is to find some satisfactory way of transforming each tendency into a number so that each karyotype can be represented in a plane by means of the rectangular coordinate system.

In *Ranunculus* morphological variations of the karyotype could be governed by symmetry. Degree of symmetry is a two component characteristic of the karyotype. The components, measured by REC and SYi indices, seem to be balanced (Fig. 5). Such balance may control chromosome rearrangements. In this case it would be reasonable to expect, for instance, that a chromosome fission would have more chances to succeed and become stabilized when it occurs in the largest median rather than in the smallest median. In the first case the acquisition of two new telocentrics (SYi index decrease) would be compensated for by a reduction of chromosome length disparity (REC index increase); in other words such rearrangement would alter the indices in opposite directions without really upsetting the balance. In the second case, the acquisition of two new telocentrics would instead increase chromosome length disparity altering both indices in the same direction. Thus, if it is true that each symmetry component can vary more freely than their sum, the prediction is that smaller medians should be more resistent to fission than the larger ones.

It seems as if in *Ranunculus* relative length and centromere position of one chromosome could affect and in turn be affected by the same properties of all the other chromosomes of the complement. LIMA DE FARIA noticed (1954) that

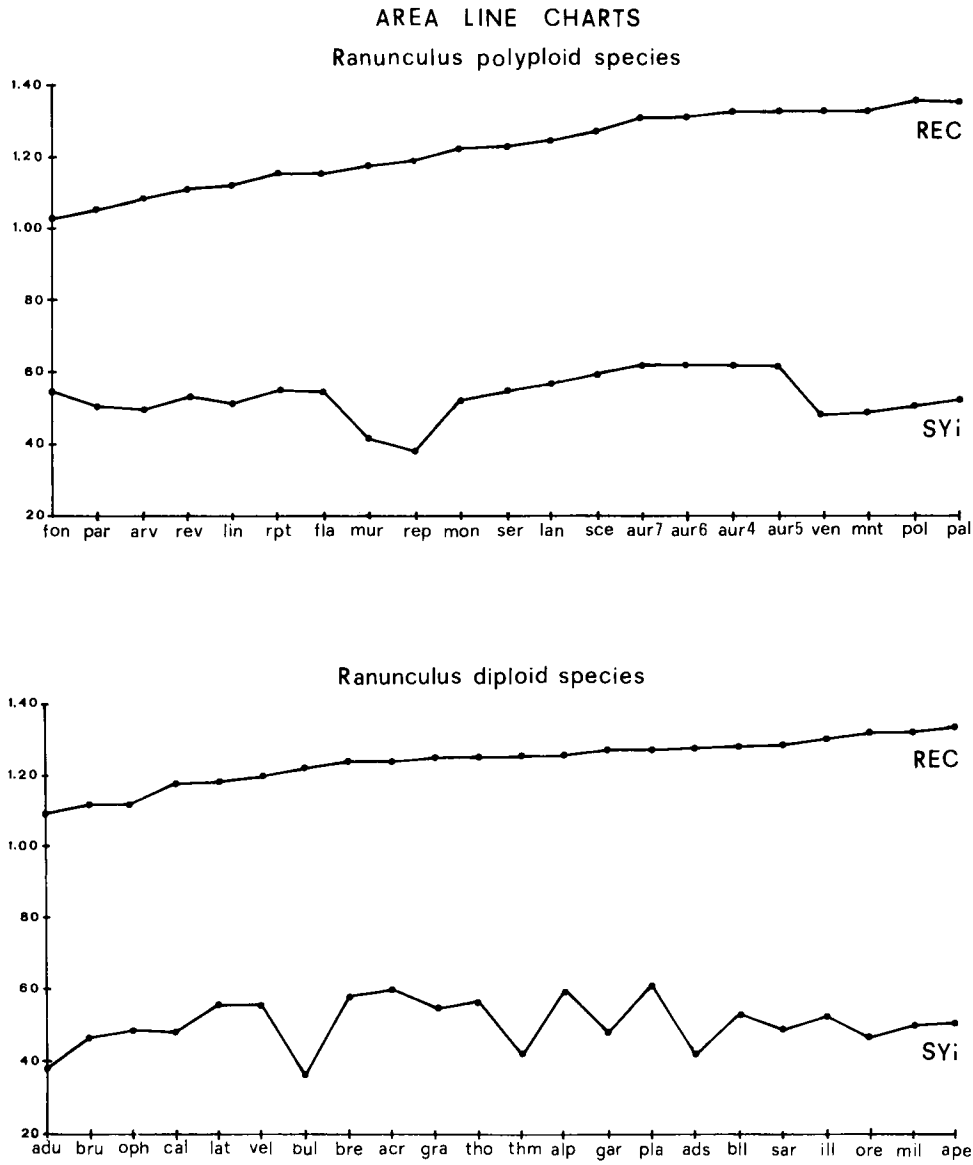

Fig. 5 — Area line chart of karyotype symmetry indices (REC and SYi) in 21 polyploid species of *Ranunculus* (above) and of 22 diploid species of *Ranunculus* (below). (REC lines are based on SYi lines rather than from the X axis). Species are ordered by increasing karyotype symmetry. For decodification of species names see the text. (Data partially from D'OVIDIO *et al.* 1985).

properties exhibited by certain entities in one part of the chromosome bear a definite relation to the properties exhibited by other entities in other parts of the chromosome, and developed the «chromosome field» concept. Our idea is that perhaps in *Ranunculus* there is not only a chromosome field but also a «karyotype field». «... there are rigid constraints upon chromosome organization during speciation. While the proliferation of the DNA sequences appears to be fortuitous, its distribution within the complement is governed by the constraints inherent to the stable organization of a genome» (NARAJAN 1987).

However constraints to chromosome organization are certainly diversified among plant groups and not always apparent. In *Scilla persica* and in seven species of the *Scilla hohenackeri* group (GREILHUBER and SPETA 1976) correlation between the indices is not significant for $p < 0.1$ and anyhow positive. Twenty species of shrubby *Oxalis* have been studied by means of the multivariate analysis of their karyotype components (chromosome number, ploidy level, 2C DNA content, chromosome volumes, average arm ratio and coefficient of interchromosomal variation) (DE AZKUE and MARTINEZ 1987). This little group shows a really unusual high variation of karyotype parameters. Species with a large amount of DNA are assumed to be derived from those with less DNA. With few exceptions, species with a small amount of DNA (50% of the examined ones) have karyotypes composed by metacentric, or nearly so, chromosomes. As the DNA content increases, karyotypes become prevalently or completely composed by telocentrics: «the unusual diversity in chromosome morphology found in shrubby *Oxalis* is mainly due to a wide variation in DNA content and the inequal distribution of the extra DNA in the chromosomes».

CONCLUSIONS

On species belonging to Sections FLAMMULA and MICRANTHUS.

Karyotype typical elements of *Ranunculus* species belonging to Sections FLAMMULA and MICRANTHUS (LANCIFOLII group) can be seen in Fig. 4 and Fig. 5. Here such elements are compared to those shown by other 32 species of *Ranunculus* (plus four polyplotypes of *R. auricomus* L. s.l.). If degree of karyotype symmetry can be measured (Fig. 4) by the distance of points representing species from the origin of the axes, then LANCIFOLII's karyotypes (area 9) are the most asymmetric among *Ranunculus* karyotypes with the only exception of Section ECHINELLA DC. species (area 8a) and, partially of *R. brutius* Ten. (area 2c). Moreover REC and SYi indices (Fig. 5) are amongst LANCIFOLII always rather near to each other. *R. fontanus* Presl karyotype is rather peculiar showing, besides the lowest REC value, the only case of a SYi value higher than the corresponding REC value.

Figs. 6-11. — Metaphase chromosomes of *Ranunculus* species. Fig. 6 — *R. ophioglossifolius* Vill., 1500×. ($2n = 2x = 16$). Fig. 7 — *R. flammula* L., 1500×. ($2n = 4x = 32$). Fig. 8 — *R. revelieri* Boreau, 1500×. ($2n = 4x = 32$). Fig. 9 — *R. reptans* L., 1500×. ($2n = 4x = 32$). Fig. 10 — *R. fontanus* Presl, 1500×. ($2n = 6x = 48$). Fig. 11 — *R. lateriflorus* DC., 1500×. ($2n = 2x = 16$).

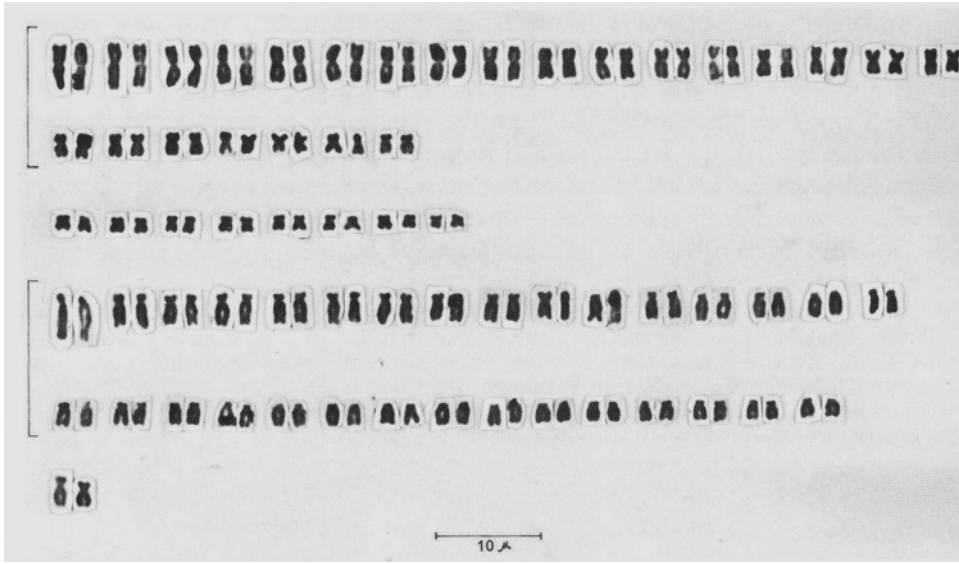

Fig. 12 — Metaphase chromosomes of *Ranunculus lingua* L., 2100×. ($2n = 16x = 128$).

On Ranunculus *L. subgen.* Ranunculus.

In *Ranunculus* microdensitometry has been used to measure variations of nuclear DNA content (karyotype size). Variations of relative chromosome length and of centromere position within each karyotype have been evaluated by karyometry and calculation of GREILHUBER and SPETA (1976) symmetry indices.

Comparison between specific karyotype structures is largely committed to diagrams. Fig. 4 and 5 were obtained plotting GREILHUBER and SPETA indices in two different manners: the scatter diagram (Fig. 4) where one point represents one species, and the area line diagram (Fig. 5) where indices corresponding to the same species are independently represented on the same vertical.

The diagram in Fig. 4 shows that in *Ranunculus* karyotype symmetry seems to be altogether inversely correlated to the evolutionary progression outlined by DAVIS (1960) on the grounds of macromorphic and ecological elements) in accordance with STEBBINS (1970) observation: «during the evolution of higher plants... probably... trends toward greater asymmetry are more common than their reversal».

Fig. 4 suggests also that certain subgeneric taxa (i.e. area 4, Subsection EPIROTES Prantl 1888) can predict better than others (i.e. areas 8a and 8b, informal group ANNUI Davis 1960) the location of species in the REC-SYi plane.

Apparently in *Ranunculus* there is a precise constraint governing karyotype structure. Fig. 5 graphs attempt to visualize the fact that in *Ranunculus*, throughout polyploid and diploid species, total karyotype symmetry is less variable than it could be expected from the separate evaluation of its components (measured here by REC and SYi indices). This suggests that possibly in this genus karyotype variation and evolution proceed by an alternation of short intervals of generalized chromosome unstability and long periods of chromosome stability rather than by accumulation of isolated mutational events.

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