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Chromosome numbers and karyotypes within the *Ranunculus alpestris*-group (Ranunculaceae)

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Abstract

The *Ranunculus alpestris*-group comprises six white-flowered species growing in mostly alpine zones of central and southern European mountains. They all are diploid with $2n = 16$ chromosomes. The common karyotype of the group was established based on 75 metaphases (6–26 metaphases per species). The haploid karyotype consists of four metacentric (chromosomes 1, 3, 6, 7) and four more or less subtelocentric chromosomes (2, 4, 5, 8). This karyotype is similar to that of other white-flowered European *Ranunculus* species as well as the yellow-flowered *R. thora*-group. Analysis of karyotypes partly confirms relationships inferred from molecular phylogenies. Species with this karyotype are placed on rather basal branches in existing phylogenies, which may indicate that this karyotype is primitive within the genus *Ranunculus*.

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Introduction

Modern biosystematic investigations are mainly based on molecular methods, but morphology and karyology remain essential information on any organism. Many karyological investigations have been performed over the last decades and provide fundamental characters for plant systematics and evolutionary analysis (Stace 2000). Despite their taxonomic relevance, chromosome numbers are known for only about 25% of all angiosperms (Bennett 1998). Moreover, many reported chromosome numbers are more or less uncertain, because they are based on poor data (from a single population or even only a single plant; not well

documented, e.g. lacking data on voucher specimens or their origins). This reduces the usefulness of the results, especially in taxonomically difficult groups in which chromosome numbers are often variable. Besides variation in chromosome number (including ploidy level and aneuploidy), karyological data can show variation in absolute and relative chromosome size, in chromosome morphology, and in staining properties of the chromosomes (Sharma and Sen 2002). The most obvious morphological character of a chromosome is the position of the centromere (Levan et al. 1964). The description of chromosome morphology has proved to be a powerful method to characterise genomes in plants but also in animals, including humans.

Chromosome data provide essential information for various fields, as illustrated by the “taxonomic importance of karyology at the generic and subgeneric level”

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in Geraniaceae (Albers and Van der Walt 2007). Karyological data may also help to interpret results from molecular studies (Johansson 1998; Schuettpelez et al. 2002; Crawford et al. 2005; Hörandl et al. 2005). Karyological data (besides morphological characters) have aided or even made possible taxonomic decisions, in tracing the origin of hybrids, e.g. in the now classic case in *Tragopogon* (Ownbey and McCollum 1954) or more recently in *Fallopia* (Bailey and Stace 1992).

Stace (2000) proposed that three main conditions must be fulfilled for chromosome numbers to be useful: the investigated plants should originate from known wild localities, voucher specimens must be deposited in a designated and accessible herbarium, and counts have to be based on several plants in each population of a given taxon. Furthermore, ideally several populations from different geographic regions should be investigated. Of special interest are marginal sites or isolated areas in the geographic distribution of the respective taxon. Invasive species may also be of special karyological interest because they may show different patterns of ploidy levels in native and introduced areas. This is the case, e.g., for *Erigeron annuus* (L.) Pers. s.l. and *Solidago gigantea* Aiton, which are both native to America and introduced in Europe and elsewhere. *Erigeron annuus* s.l. consists of diploid to tetraploid individuals in America, but only triploids are found in Europe (Frey et al. 2003); *Solidago gigantea* is diploid to hexaploid in America, but only strongly invasive tetraploids occur in Europe and East Asia (Schlaepfer et al. 2008a, 2008b). *Centaurea maculosa* Lam. and *C. diffusa* Lam. are mostly diploid in Europe where they are native, but are invasive and exclusively tetraploid in America (Müller 1989; Müller-Schärer 1993).

Ranunculaceae is a medium-sized family with many primitive characters but also presents specialised and advanced features. Karyological characters are considered to be of great importance in the phylogenetic classification of Ranunculaceae (Tamura 1995). *Ranunculus* is the largest genus in the family, with about 600 species and a world-wide distribution, but most species grow in temperate to arctic zones (Tamura 1993). The chromosome number in *Ranunculus* usually is $x = 7$ or $x = 8$, with the latter being much more frequent and regarded as the basic chromosome number (Goepfert 1974). Polyploidy is frequent, and variation in ploidy levels may even occur within species (Küpfer 1974; Vuille and Küpfer 1985; Baack 2004; Baltisberger and Widmer 2005; Rossello and Castro 2008). Karyotypes vary considerably within the genus (Goepfert 1974), and even species that are not closely related can hybridize, at least under experimental conditions (Goepfert 1975; Baltisberger 1981; Huber 1988). Therefore, hybridization and polyploidy may play an important role in speciation in *Ranunculus* (Baack 2005; Hörandl et al. 2005).

The *Ranunculus alpestris* species group is characterised by small, mostly one-stemmed plants with one to two (very rarely more) flowers, petiolate basal leaves with a more or less lobed suborbicular or orbicular blade, and by white honey-leaves. The group consists of seven taxa occurring in central and southern European mountain ranges (Jalas and Suominen 1989): *R. alpestris* L. (frequent throughout the Alps, but also in the Jura, Pyrenees, Carpathians, and N Appenini), *R. bilobus* Bertol. (S Alps), *R. cacuminis* Strid & Papanicolaou (Mt. Kajmakalan in Greece/Macedonia), *R. crenatus* Waldst. & Kit. (Carpathians, E Alps, Balkan Peninsula), *R. magellensis* Ten. (C Appenini), *R. traunfellneri* Hoppe (SE Alps), and the infraspecific taxon *R. alpestris* ssp. *leroyi* Lainz (Cordillera Cantabrica; Lainz and Loriente 1981).

Ranunculus alpestris and *R. crenatus* are widespread, the others are more or less narrow endemics. The plants of all taxa grow in the alpine or rarely subalpine zones on meadows, gravels, and rocky ledges. *Ranunculus cacuminis* and *R. crenatus* are calcifuge, the others calcicole. The taxa are mainly distinguished by the shape (depth of incisions, leaf basis) and venation of the basal leaves, which is visually striking but taxonomically a poor indicator of relationships within *Ranunculus*. Additional characters to distinguish the taxa are the nectary scales and the size of the achenes. The group can be divided into two subunits (Müller and Baltisberger 1984; Baltisberger 1994): *R. alpestris* (including ssp. *leroyi*), *R. bilobus* and *R. traunfellneri* have no nectary scale, small achenes, and a strongly visible leaf venation, whereas *R. cacuminis*, *R. crenatus* and *R. magellensis* show well developed nectary scales, bigger achenes, and an obscure venation on the leaves. This division is supported by molecular data (Paun et al. 2005; Widmer unpubl. data).

Four species in the group are self-incompatible and obligate outcrossers; only *R. crenatus* and *R. magellensis* are partly self-fertile (Müller and Baltisberger 1984; Baltisberger 1994). The morphological variability of the outcrossing *R. alpestris* is rather pronounced; many varieties and subspecies have been described but have no taxonomic value (Kunz 1938). *Ranunculus bilobus* and *R. traunfellneri* are very closely related to *R. alpestris* and can be considered as two extreme forms in a morphological continuum with *R. alpestris* as the midzone. Compared to *R. alpestris*, *R. bilobus* and *R. traunfellneri* both show less morphological variability and grow in clearly separated, marginal geographic areas. They are therefore treated as separate species. The morphological differences between *R. alpestris* s.str. and *R. alpestris* ssp. *leroyi* are weak; the validity of the subspecies is questionable. The results of ongoing molecular investigations may shed further light on this question.

In the course of our studies on the *Ranunculus alpestris*-group over the last years, a lot of data have

accumulated, e.g. on karyology, crossing experiments, isozyme analysis, and variation in DNA sequences. Last summer, on a field trip with students, we discovered at a natural site in the alpine zone some dozen gigantic individuals of *R. alpestris* with up to five flowers per plant (Fig. 1). These abnormal plants were scattered in



Fig. 1. *Ranunculus alpestris* from the region of Davos, Grisons, Switzerland (2300 m a.s.l.). (A) Gigas form. (B) Normal size.

a huge population with thousands of individuals of normal *R. alpestris*. The question arose whether these plants are polyploids. As polyploid individuals were unknown from the species group, we sampled seven live plants and investigated them karyologically. We present the results here, together with data from earlier investigations, and review the karyology of the entire *R. alpestris*-group.

Material and methods

Living plants were collected from natural habitats and transported to Zürich. Whenever possible, herbarium specimens of plants from the natural site as well as from cultivation in the greenhouse were sampled and deposited in Z/ZT (herbarium of ETH and University of Zürich). The sites investigated cover the whole area of geographic distribution for all taxa (Fig. 2).

The karyological investigations were carried out on root tips. These were pretreated with colchicine (0.05%) for 2 hours, fixed in ethanol/acetic acid (3:1), then stained and squashed in lacto-propionic orcein (Dyer 1963). To determine chromosome numbers, 5–10 metaphases were counted out of each individual investigated, and several individuals per site were examined (Table 1).

To investigate karyotypes in detail, good metaphases were drawn with a camera lucida. The size of the chromosomes in the drawings varied from 0.5 to 2 cm. Each chromosome arm was measured separately to an accuracy of 0.25 mm. The resulting values were

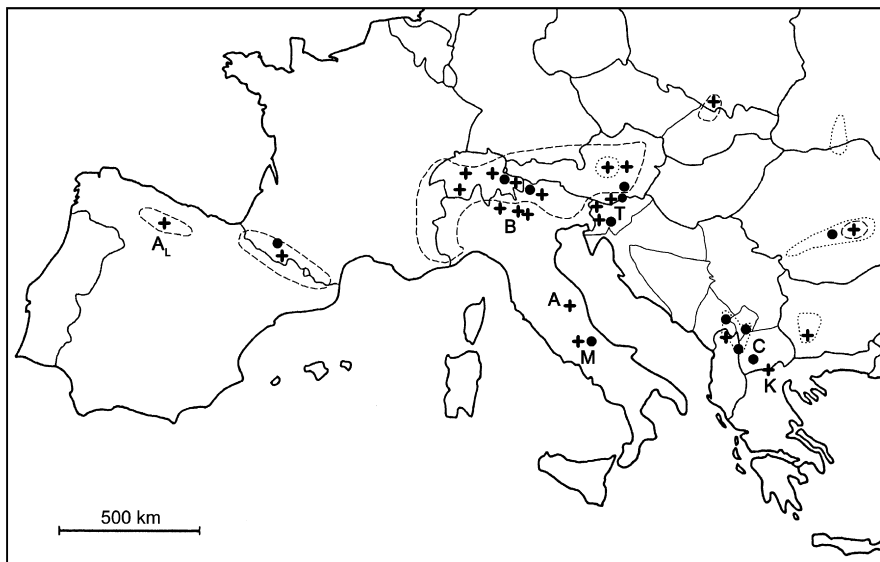


Fig. 2. Distribution map of the *Ranunculus alpestris*-group (based on Jalas and Suominen 1989; Baltisberger 1994), showing locations of sampling sites (+ = karyotype; ● = chromosome number); each individual symbol may represent more than a single site. Localities A and areas enclosed by dashed lines = *R. alpestris* (A_L = *R. alpestris* ssp. *leroyi*); B = *R. bilobus*; C and areas enclosed by dotted lines = *R. crenatus*; K = *R. cacuminis*; M = *R. magellensis*; T = *R. traunfellneri*.

Table 1. List of investigated plant specimens, showing respective sampling site, sampling year, voucher numbers of herbarium specimens deposited in Z/ZT, number of individuals investigated for chromosome numbers (N), and number of metaphases measured for karyotypes (KT).

Taxon and site	Year	Specimen numbers	N	KT
<i>Ranunculus alpestris</i>				
Arnifirst, Obwalden, CH	1975	81/1002	+	8
Planneralm, Steiermark, A	1992	12621, 13170	11	1
Gemmi, Valais, CH	1992	13627, 13186	9	1
Passo le Vette Grande, Feltre, I	1992	12688, 13140	*	1
Krzesanica, Tatra, PL	1992	Z/S-Tatra, 13174	11	5
Ofenpass, Grisons, CH	1995	13050, 13172	7	–
Monte Vettore, Monti Sibillini, I	1995	14409, 13263	*	1
Cirque de Troumouse, Haut-Pyrénées, F	1995	14420, 13168	11	1
Piatra Craiului, Carpathians, RO	1997	13354	10	1
Fuorcla Laschadurella, Grisons, CH	1997	13384, 13474	9	2
Piz Umbrail, Grisons, CH	1997	13410, 13497	7	1
Pizol, St. Gall, CH	2000	13580, 13807	11	1
Rollspitze, Südtirol, I	2000	13628, 13762	9	–
Cimon della Pala, Dolomiten, I	2000	13629, 13769	9	1
Schiahorn, Davos, Grisons, CH	2007	13939	7	–
<i>Ranunculus alpestris</i> ssp. <i>leroyi</i>				
Picos de Europa, E	2001	01-0019, 13900	8	2
<i>Ranunculus bilobus</i>				
Passo Tremalzo, Trento/Brescia, I	1976	80/1870	°	8
Passo Tremalzo, Trento/Brescia, I	1992	12694, 13134	*	1
Cime Baremone, Brescia, I	1992	12695, 13173	*	1
Monte Alben, Alpi Bergamaschi, I	1992	12704, 13132	*	1
<i>Ranunculus cacuminis</i>				
Kajmakcalan, Voras Oros, GR	1987	11419	§	6
<i>Ranunculus crenatus</i>				
Planneralm, Steiermark, A	1976	[78/840]	°	9
Bösenstein, Steiermark, A	1992	12616, 13262	11	1
Planneralm, Steiermark, A	1992	12620, 13171	11	1
Musala, Rila Mountains, BG	1993	12739, 13178	"	1
Mt. Tarcu, Carpathians, RO	1997	13401, 14006	10	–
Maja e Jezercës, Shkodër/Tropojë, AL	2000	13891	5	1
<i>Ranunculus magellensis</i>				
Monte Sirente, Abruzzi Mountains, I	1978	80/1876	°	7
Monte Sirente, Abruzzi Mountains, I	1993	12831, 13175	*	1
<i>Ranunculus traunfellneri</i>				
Bärental, Kärnten, A	1976	no specimen	°	9
Koschuta, Kärnten, A	1992	12658, 13137	11	–
Vrsic, SLO	1992	12671, 13136	11	1
Cima Piccola, Monte Mangart, SLO	1992	12675, 13135	11	1

References for chromosome numbers: + = Baltisberger and Müller (1981); ° = Müller and Baltisberger (1984); § = Baltisberger (1994); * = Baltisberger et al. (1996); " = Baltisberger (2006).

transformed to relative lengths expressed in % of the respective total complement length. As satellites were not always visible they were not included in the total lengths.

The karyotypes of all seven taxa in the group have been investigated. The data on 8 metaphases of *R. alpestris* and 6 metaphases of *R. cacuminis*

were published earlier (Baltisberger and Müller 1981; Baltisberger 1994). Karyotypes of *R. bilobus* (based on 8 metaphases), *R. crenatus* (9), *R. magellensis* (7), and *R. traunfellneri* (9) were discussed briefly in Müller and Baltisberger (1984), without providing the numerical data. Additionally, metaphases of all taxa except

R. cacuminis were investigated during the last years. All these data are included in the dataset presented here; the numbers of metaphases measured per site are indicated in Table 1. To discuss the results we use the terminology for chromosome morphology proposed by Levan et al. (1964). In this system, chromosomes are named according to centromere position, which is determined by the arm ratio $R = LA/SA$ (length of long arm/length of short arm). The following chromosome types are distinguished: metacentric (abbreviation M; arm ratio $R = 1.0–1.7$), submetacentric (SM; $R = 1.7–3.0$), subtelocentric (ST; $R = 3.0–7.0$), and acrocentric (A; $R > 7.0$).

Results

Chromosome numbers

The chromosomes of 163 plants (85 *Ranunculus alpestris* s. str., 8 *R. alpestris* ssp. *leroyi*, 37 *R. crenatus*, and 33 *R. traunfellneri*) were counted (Table 1). All plants showed the same diploid chromosome number $2n = 2x = 16$.

Karyotypes

In total our dataset (Table 2) consists of 75 metaphases (24 of *R. alpestris* s.str., 2 of *R. alpestris* ssp. *leroyi*, 11 of *R. bilobus*, 6 of *R. cacuminis*, 13 of *R. crenatus*, 8 of *R. magellensis*, 11 of *R. traunfellneri*). All showed the same type of chromosomes. The eight chromosomes of the haploid karyotype can be distinguished morphologically by size and arm ratio (Fig. 3). Four of them (chromosome numbers 1, 3, 6, 7) are metacentric (M) with an arm ratio between 1.06 and 1.39. Two of these (1, 3) are large and differ in about 10% of their length, the remaining two (6, 7) are smaller. The short arm of chromosome 6 is shorter, the arm ratio higher than in chromosome 7. Chromosomes 2 and 4 show an arm ratio between 2.45 and 3.05, which is in the range of the border between submeta- and subtelocentric (SM/ST). They differ in size (about 20%). Chromosome 5 is subtelocentric (ST) with an arm ratio between 3.47 and 5.90. The smallest chromosome (number 8) has an arm ratio between 5.03 and 8.75, which is in the range of the border between subtelocentric and acrocentric (ST/A). This chromosome bears a satellite (SAT) that is a bit larger than the short arm but nevertheless not always visible.

Discussion

Chromosome numbers

All taxa in the *Ranunculus alpestris*-group are diploid and show $2n = 16$ chromosomes. This agrees with all

previous records in the literature (Table 3; several compilations referred to Rațiu 1982 for a chromosome count on *R. alpestris*, but no such data are found in that work). The chromosome number of *R. alpestris* ssp. *leroyi* was not mentioned explicitly in the literature, but as the material investigated by K upfer (1974) and Diosdado and Pastor (1993) had originated from Picos de Europa, it most likely represented that taxon. The group is karyologically homogenous. Even the giant individuals from Davos (see Fig. 1) proved to be diploid, not polyploid as presumed. The reason why these plants are so tall is unknown. They grew among normal-sized individuals, with no apparent difference between the respective habitats.

Members of six different groups of *Ranunculus* occur mainly or exclusively in the alpine zone of the European mountains. Two additional species, the diploid *R. glacialis* L. and *R. pygmaeus* L., grow at high alpine sites in the Alps. Two of those groups (*R. montanus* Willd. s.l., *R. thora* L. s.l.) as well as *R. pygmaeus* are yellow-flowered, the remainder are white-flowered like *R. alpestris* s.l.

With respect to chromosome number and ploidy level, the *R. alpestris*-group is distinct from four of the six alpine groups. Only the white-flowered *R. aconitifolius* L. s.l. (for compilation see Huber 1988) and the yellow-flowered *R. thora* s.l. (see Baltisberger 1990b) are also karyologically homogenous at the diploid level. The remaining groups all contain at least two ploidy levels each. Diploid and tetraploid taxa occur in *R. montanus* s.l. (Landolt 1954) and *R. parnassifolius* L. s.l. (see Huber 1988). The group of *R. pyrenaicus* L. contains the even more variable *R. kuepferi* Greuter & Burdet with diploid, triploid, tetraploid, and pentaploid plants (Huber 1988). A special case is the group of *R. sequieri* Vill.: *R. sequieri* is diploid and widespread (scattered in the Alps and central Appenini), whereas the remaining two taxa are rare narrow endemics occurring far from the Alps. *Ranunculus sequieri* ssp. *cantabricus* Rivas Martinez & al. from the Cordillera Cantabrica is also diploid, but *R. montenegrinus* (Hal.) Lindtner, known from three sites in the Balkans, is polyploid, i.e. pentaploid at one site in Montenegro, tetraploid at two sites in Macedonia (Baltisberger 1990a, 1992).

It seems that there is no general pattern for alpine *Ranunculus* species regarding karyological homogeneity and geographic distribution. Polyploids can be isolated from the area of the related diploid taxon (e.g. *R. montenegrinus* far away from the diploid *R. sequieri*), or they can be sympatric with diploids (but probably separated ecologically), as in several combinations within *R. montanus* s.l. On the other hand, taxa with an isolated geographic area can also be diploid like the widespread relative (e.g. *R. sequieri* ssp. *cantabricus*). The widespread distribution area of a purely diploid group can be more or less continuous (as in

Table 2. Relative chromosome length (LA = long arm; SA = short arm; SAT = satellites) in % of total complement length, and arm ratio R = LA/SA in species and subspecies of the *Ranunculus alpestris*-group.

	N	Chromosome 1			Chromosome 2			Chromosome 3			Chromosome 4			Chromosome 5			Chromosome 6			Chromosome 7			Chromosome 8			SAT
		LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	
<i>R. alpestris</i> s.str.	24	4.58 0.30	4.07 0.28	1.13 0.08	4.63 0.37	1.72 0.16	2.71 0.27	4.26 0.25	3.89 0.25	1.10 0.05	3.92 0.27	1.37 0.18	2.91 0.42	3.79 0.32	0.79 0.13	4.94 0.78	3.68 0.31	2.80 0.18	1.32 0.10	3.51 0.22	3.23 0.19	1.09 0.06	3.28 0.30	0.49 0.11	6.94 1.44	0.66 0.13
<i>R. alpestris</i> ssp. <i>leroyi</i>	2	4.62 0.25	4.38 0.27	1.06 0.07	4.50 0.03	1.76 0.10	2.56 0.14	3.95 0.10	3.65 0.09	1.08 0.03	4.07 0.25	1.58 0.15	2.58 0.18	3.77 0.28	0.85 0.12	4.50 0.67	4.03 0.68	2.93 0.59	1.38 0.10	3.34 0.35	2.92 0.28	1.15 0.04	3.16 0.35	0.49 0.01	6.50 0.82	0.85 0.14
<i>R. bilobus</i>	11	4.67 0.26	3.88 0.30	1.21 0.08	4.56 0.21	1.75 0.18	2.62 0.27	4.29 0.25	3.90 0.22	1.10 0.05	3.94 0.25	1.32 0.18	3.05 0.49	3.87 0.21	0.68 0.14	5.90 1.20	3.68 0.24	2.89 0.25	1.28 0.09	3.45 0.17	3.24 0.21	1.07 0.05	3.45 0.15	0.44 0.14	8.75 3.04	0.70 0.17
<i>R. cacuminis</i>	6	4.83 0.23	3.98 0.30	1.22 0.12	4.83 0.31	1.61 0.19	3.04 0.33	4.53 0.26	4.18 0.22	1.08 0.05	3.90 0.24	1.34 0.18	2.96 0.45	3.62 0.28	1.05 0.10	3.47 0.41	3.41 0.30	2.50 0.43	1.39 0.19	3.53 0.28	3.08 0.24	1.15 0.08	3.00 0.19	0.62 0.10	5.03 1.10	0.69 0.10
<i>R. crenatus</i>	13	4.81 0.22	4.08 0.31	1.18 0.09	4.72 0.24	1.83 0.15	2.59 0.22	4.34 0.29	4.00 0.28	1.09 0.06	3.84 0.21	1.39 0.17	2.80 0.37	3.70 0.24	0.78 0.17	4.94 1.14	3.47 0.28	2.78 0.23	1.25 0.11	3.42 0.24	3.12 0.21	1.10 0.06	3.24 0.22	0.48 0.11	7.12 1.47	0.58 0.11
<i>R. magellensis</i>	8	4.72 0.31	4.05 0.21	1.17 0.07	4.57 0.27	1.88 0.19	2.45 0.24	4.26 0.13	4.01 0.15	1.07 0.04	3.80 0.31	1.47 0.20	2.63 0.44	3.76 0.23	0.87 0.07	4.32 0.44	3.49 0.32	2.78 0.20	1.26 0.11	3.46 0.25	3.14 0.30	1.11 0.06	3.17 0.14	0.56 0.07	5.72 0.70	0.75 0.19
<i>R. traunfellneri</i>	11	4.60 0.17	4.00 0.21	1.15 0.07	4.59 0.18	1.80 0.16	2.57 0.22	4.27 0.15	3.96 0.15	1.08 0.05	3.92 0.27	1.36 0.16	2.91 0.35	3.80 0.23	0.70 0.10	5.51 0.80	3.66 0.22	2.79 0.19	1.32 0.12	3.55 0.13	3.31 0.20	1.07 0.05	3.23 0.20	0.47 0.10	7.15 1.52	0.59 0.14
Our common karyotype	75	4.67 0.27	4.03 0.28	1.16 0.09	4.63 0.29	1.77 0.18	2.65 0.29	4.30 0.25	3.95 0.24	1.09 0.05	3.90 0.26	1.38 0.18	2.88 0.42	3.77 0.27	0.79 0.16	4.97 1.07	3.61 0.32	2.79 0.26	1.30 0.12	3.48 0.22	3.19 0.23	1.09 0.06	3.25 0.25	0.50 0.12	6.97 1.95	0.66 0.15
Chromosome type	M				SM / ST			M			SM / ST		ST		M			M		M			ST / A		SAT	

N = number of metaphases measured. Respective upper value = average, lower value = standard deviation. For chromosome types, see text on material and methods.

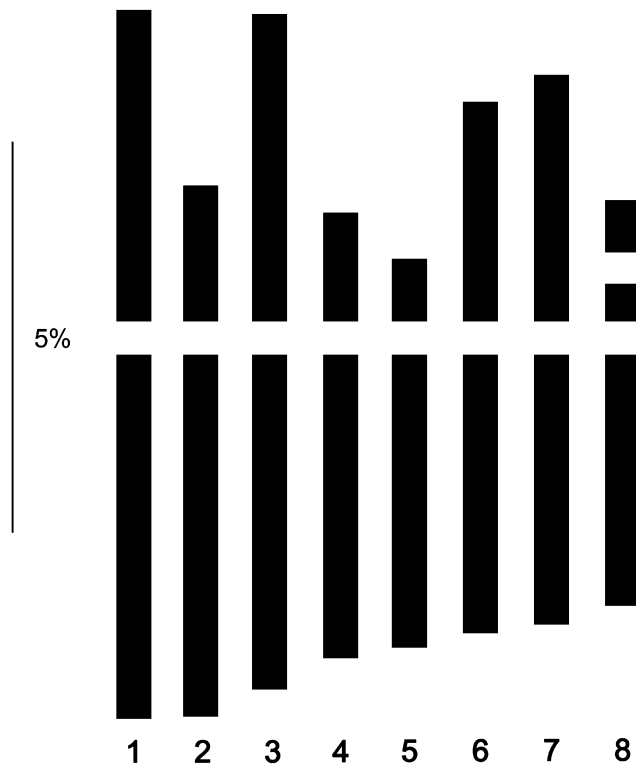


Fig. 3. Karyogram of the *Ranunculus alpestris*-group based on 75 metaphases from all taxa in the group. Chromosomes arranged by decreasing size of the long arm; size corresponds to relative length of total complement length (2x).

R. aconitifolius s.l.) or disjunct (as in *R. thora* s.l.). The group of *R. alpestris* contains strongly isolated narrow endemics (e.g. *R. alpestris* ssp. *leroyi* and *R. cacuminis*) and two widespread species: *R. alpestris* s.str. with a mostly continuous geographic area (but also isolated marginal populations), and *R. crenatus* with very disjunct areas. No deviating chromosome number (neither polyploid nor aneuploid) has been detected in any area or taxon of the *R. alpestris*-group.

Karyotypes within *Ranunculus alpestris* s.l.

The borders between chromosome types as defined by the arm ratio (meta-, submeta-, subtelo-, and acrocentric; Levan et al. 1964) are arbitrary and in some cases impractical. For any given chromosome, arm ratios from several plants can fall right on such a border with little variation to either side. This can result in different chromosome-type assignments for metaphases from one taxon or even from a single plant. This is the case with three chromosomes (numbers 2, 4, 8) in the complement of *Ranunculus alpestris* s.l. Neglecting these terminological differences, the karyotypes of taxa in the *R. alpestris*-group show no significant variation. Therefore, we pooled all 75 metaphases investigated and

calculated the common karyotype for all taxa in the group (Table 2; Fig. 3).

Bauer (1954) and Goepfert (1974) gave karyotypes based on measurements but used different modes of presenting the numbers. We transformed their numbers into the mode we used here (Table 4). Neither author indicated the numbers of metaphases measured or standard deviations for their numbers; thus, nothing can be said about the soundness of their data or the variability of the karyotypes presented. The latter fit our karyotype well, and most of the chromosomes agree with the ones we found. However, the following differences can be observed: Bauer (1954) indicated chromosome 6 in *R. alpestris* to be submetacentric (not metacentric), and chromosome 4 as relatively small, but chromosome 5 as relatively large (compared to our data). The karyotype of *R. alpestris* in Goepfert (1974) corresponds to the one we found, but in the karyotypes of the other two species one chromosome each differs from our data: chromosome 2 in *R. crenatus* and chromosome 4 in *R. traunfellneri* were both indicated to be subtelo- (instead of submetacentric).

Several papers gave similar karyotypes for various taxa in the *Ranunculus alpestris*-group, but without presenting measurement results. Gregson (1965) indicated the same karyotype as ours for *R. magellensis* (including the satellites of the smallest chromosome). Sopova and Sekovski (1982) gave a similar karyotype for *R. crenatus* but without satellites on the smallest chromosome, as did Diosdado and Pastor (1993) for *R. alpestris* originating from the Cantabrian mountains (thus probably representing ssp. *leroyi*). As the satellites are not visible in all metaphases in those works, it is conceivable that they had been overlooked. The karyotypes given in Baltisberger et al. (1996; for *R. alpestris*, *R. bilobus*, and *R. magellensis*) and Baltisberger (2006; *R. crenatus*) agree with our karyotype of the group. The karyotype data in Baltisberger and Müller (1981; *R. alpestris*), Müller and Baltisberger (1984; *R. bilobus*, *R. crenatus*, *R. magellensis*, and *R. traunfellneri*), and Baltisberger (1994; *R. cacuminis*) are included in the present dataset.

Phylogenetic relevance of karyotypes

Based on chromosome number and shape, Langlet (1932) recognised several groups in the family Ranunculaceae and even within the genus *Ranunculus*. Bauer (1954) was the first to present quantitative data on chromosome morphology. She investigated three white-flowered taxa occurring in the Polish Tatra Mts.: *R. alpestris*, *R. glacialis*, and *R. platanifolius*. In his extensive study, Goepfert (1974) characterised more than 100 species of *Ranunculus* by chromosome morphology. Some sections showed a homogenous

Table 3. Overview of literature references to karyological data ($n = x = 8$ or $2n = 2x = 16$).

Reference	Origin of plants	KT
Langlet (1936) ^a	unknown	–
<i>Ranunculus alpestris</i>		
Langlet (1927)	unknown (botanical garden)	–
Bauer in Skalinska (1950)	3 x Poland	–
Mattick in Tischler (1950)	unknown	–
Bauer (1954)	11 x Poland	+
Larsen (1954)	2 x Switzerland	–
Sušnik (1961)	? x Slovenia	–
Gregson (1965)	1 x Switzerland	–
Goepfert (1974)	4 x unknown	+
Küpfer (1974)	1x France	–
Vachova in Majovsky et al. (1974)	1 x Slovakia	–
Baltisberger and Müller (1981)	1 x Austria, 2 x Switzerland	+
Marchi and Visona (1982)	1 x Italy	–
Müller and Baltisberger (1984)	1 x each Austria, France, Italy, Switzerland	–
Murin (1986)	1 x Slovakia	–
Huber (1988)	3 x Switzerland	–
Baltisberger in Stace (1991)	1 x Switzerland	–
Baltisberger et al. (1996)	2 x Italy	+
<i>Ranunculus alpestris</i> ssp. <i>leroyi</i>		
Küpfer (1974) ^b	1 x Spain	–
Diosdado and Pastor (1993) ^b	2 x Spain	+
Diosdado and Pastor (1996) ^b	(2 x Spain; same counts as in Diosdado and Pastor 1993)	–
<i>Ranunculus bilobus</i>		
Baltisberger and Müller (1981)	1 x Italy	–
Müller and Baltisberger (1984)	4 x Italy	+
Baltisberger et al. (1996)	4 x Italy	+
<i>Ranunculus cacuminis</i>		
Baltisberger (1993)	1 x Greece/Macedonia	–
Baltisberger (1994)	1 x Greece/Macedonia, 2 x Greece	+
Matevski et al. (1996)	1 x Macedonia	–
<i>Ranunculus crenatus</i>		
Goepfert (1974)	1 x unknown	+
Baltisberger and Müller (1981)	1 x Austria	–
Sopova and Sekovski (1982)	1 x Macedonia	+
Müller and Baltisberger (1984)	1 x Macedonia, 1 x Montenegro	+
Baltisberger and Huber in Löve (1987)	1 x Austria	–
Baltisberger (1990a)	1 x Macedonia	–
Baltisberger (1992)	1 x Albania, 4 x Macedonia	–
Baltisberger (2006)	1 x Bulgaria	+
<i>Ranunculus magellensis</i>		
Gregson (1965)	1 x Italy	+
Chichiricco et al. (1979)	1 x Italy	–
Müller and Baltisberger (1984)	3 x Italy	+
Baltisberger et al. (1996)	2 x Italy	+
<i>Ranunculus traunfellneri</i>		
Böcher (1938)	unknown (botanical garden)	–
Sušnik (1961)	? x Slovenia	–
Sušnik (1962)	1 x Austria, 4 x Slovenia	–
Lovka et al. in Löve (1972)	2 x Slovenia	–
Sušnik et al. in Löve (1972)	1 x Slovenia	–
Goepfert (1974)	1 x unknown	+
Baltisberger and Müller (1981)	1 x Austria	–
Müller and Baltisberger (1984)	1 x Austria, 2 x Slovenia	+
Dobes et al. (1997)	1 x Austria	–

+ : karyotype (KT) information included.

^aTaxonomic affinity uncertain.^bUnder the name *R. alpestris*.

Table 4. Karyotypes (KT) of white-flowered European alpine species (top four rows: species in *Ranunculus alpestris*-group; fifth row: our common karyotype of the whole *R. alpestris*-group; bottom eight rows: species outside the *R. alpestris*-group): Relative chromosome length (LA = long arm; SA = short arm) in % of total complement length; R = arm ratio LA/SA.

Species (KT reference)	Chromosome 1			Chromosome 2			Chromosome 3			Chromosome 4			Chromosome 5			Chromosome 6			Chromosome 7			Chromosome 8		
	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R	LA	SA	R
<i>R. alpestris</i> (Bauer 1954)	4.61	4.61	1.00	4.94	2.20	2.25	3.96	3.96	1.00	3.23	1.32	2.45	4.29	0.99	4.33	3.96	1.71	2.31	3.63	2.64	1.38	3.30	0.66	5.00
<i>R. alpestris</i> (Goepfert 1974)	4.71	4.29	1.10	4.09	1.41	2.90	4.19	3.81	1.10	3.97	1.53	2.60	3.94	1.06	3.70	3.27	2.73	1.20	3.50	3.50	1.00	2.99	0.51	5.80
<i>R. crenatus</i> (Goepfert 1974)	4.71	4.29	1.10	4.54	0.96	4.70	4.19	3.81	1.10	4.38	1.62	2.70	3.74	0.76	4.90	3.40	3.10	1.10	3.14	2.86	1.10	3.35	0.65	5.20
<i>R. traunfellneri</i> (Goepfert 1974)	4.71	4.29	1.10	4.46	1.54	2.90	4.19	3.81	1.10	3.91	1.09	3.60	3.65	0.85	4.30	3.40	3.10	1.10	3.14	2.86	1.10	3.33	0.67	5.00
Our common karyotype	4.67	4.03	1.16	4.63	1.77	2.65	4.30	3.95	1.09	3.90	1.38	2.88	3.77	0.79	4.97	3.61	2.79	1.30	3.48	3.19	1.09	3.25	0.50	6.97
<i>R. aconitifolius</i> (Goepfert 1974)	4.19	3.81	1.10	4.33	1.67	2.60	4.09	3.41	1.20	4.33	1.17	3.70	4.12	0.88	4.70	3.55	2.95	1.20	3.27	2.73	1.20	3.96	0.54	7.40
<i>R. glacialis</i> (Bauer 1954)	4.78	4.78	1.00	4.41	2.21	2.00	4.41	3.68	1.20	3.68	1.47	2.50	3.68	0.74	5.00	3.68	1.84	2.00	3.31	3.31	1.00	3.31	0.74	4.50
<i>R. glacialis</i> (Goepfert 1974)	4.75	4.75	1.00	4.33	1.67	2.60	4.00	4.00	1.00	3.54	0.96	3.70	3.58	0.92	3.90	4.09	3.41	1.20	3.14	2.86	1.10	3.26	0.74	4.40
<i>R. plataniifolius</i> (Bauer 1954)	4.78	4.78	1.00	3.82	2.23	1.71	4.33	3.57	1.21	5.41	1.59	3.40	3.82	0.64	6.00	3.18	2.04	1.56	3.18	3.18	1.00	2.80	0.64	4.40
<i>R. parnassifolius</i> A (Goepfert 1974)	4.36	3.64	1.20	5.00	2.00	2.50	4.00	4.00	1.00	3.65	1.35	2.70	3.15	0.85	3.70	3.67	3.33	1.10	3.55	2.95	1.20	2.84	0.66	4.30
<i>R. parnassifolius</i> B (Goepfert 1974)	4.75	4.75	1.00	4.60	1.40	3.30	3.93	3.57	1.10	3.97	1.53	2.60	3.91	1.09	3.60	3.39	1.61	2.10	3.67	3.33	1.10	3.23	0.77	4.20
<i>R. sequieri</i> (B. and M. 1981)	4.77	4.09	1.17	4.54	1.66	2.73	4.31	3.99	1.08	3.96	1.57	2.52	3.77	0.80	4.71	3.49	2.56	1.37	3.57	3.36	1.06	3.06	0.49	6.22
<i>R. sequieri</i> (Baltisberger 1990b)	4.58	4.09	1.12	4.58	1.84	2.50	4.58	4.09	1.12	3.79	1.55	2.44	3.54	0.82	4.31	3.58	2.48	1.45	3.60	3.39	1.06	2.90	0.60	4.85

Data transformed from the respective reference, except for original data from Baltisberger and Müller (1981; quoted as “B. and M. 1981” above) and from Baltisberger (1990b).

karyotype, while others were split into groups based on karyotype data. The haploid karyotype with 4 metacentric and 4 more or less subtelo-centric chromosomes occurs only in a few species that are rather dissimilar at least in parts. It is interesting that in Hörandl et al. (2005; based on ITS data) and Paun et al. (2005; based on ITS, matK and trnK) the ‘white-flowered European alpiners’ (clades IX and X in the former, II and III in the latter study) with *R. alpestris* and *R. bilobus* show the same karyotype as we found in the *R. alpestris* group. These white-flowered *Ranunculus* species showing similar karyotypes as the *R. alpestris*-group are *R. aconitifolius* s.l. (Bauer 1954; Goepfert 1974; Sopova and Sekovski 1982; Diosdado and Pastor 1993), *R. parnassifolius* s.l. (Goepfert 1974; Küpfer 1974), *R. pyrenaicus* s.l. (Küpfer 1974), *R. sequieri* s.l. (Baltisberger and Müller 1981; Baltisberger 1990b, 1992; Diosdado and Pastor 1993), and *R. glacialis* (Bauer 1954; Goepfert 1974; Diosdado and Pastor 1993). The numerical data on karyotypes given by some authors for several of these species correspond to our karyotype of *R. alpestris* s.l. (Table 4).

So far, there is no molecular phylogeny that includes all members of the *R. alpestris*-group. Hörandl et al. (2005) as well as Paun et al. (2005) included *R. alpestris*, *R. bilobus*, *R. crenatus* in their phylogenies, Paun et al. (2005) also *R. traunfellneri*. In both papers, *R. crenatus* is separated from the remaining taxa in the *R. alpestris*-group. Based on molecular data, Paun et al. (2005) excluded *R. crenatus* from the *R. alpestris*-group; Paun et al. (2008) then limited the latter to the three calcifuge species *R. alpestris*, *R. bilobus*, and *R. traunfellneri*. Obviously, these three fully interfertile taxa are closely related (Müller and Baltisberger 1984) and show remarkable genetic variability. The pattern can be explained in part with geographic distribution areas and the history of speciation and migration (Paun et al. 2008). Although *R. crenatus* and probably also *R. cacuminis* and *R. magellensis* do not belong to the core *R. alpestris*-group, hybrids between members of the two units can be obtained nonetheless (Müller and Baltisberger 1984; Baltisberger 1994).

Hybrids and distribution patterns

Within these groups with similar karyotypes, hybrids can occur between taxa even if the latter differ substantially in morphology (Huber 1988), e.g. *Ranunculus* × *lacerus* Bell. (= *R. aconitifolius* × *R. kuepferi*) or *R.* × *yvesii* Burnat (= *R. kuepferi* × *R. sequieri*). Although morphologically very distinct, all these taxa are closely related phylogenetically (Paun et al. 2005). It is interesting to note that although the karyotypes seem compatible, the *R. alpestris*-group is strongly isolated from these other species, as no hybrids are known with species from other groups. In crossing

experiments involving taxa of *R. alpestris* s.l. as one parent and *R. sequieri* (see Baltisberger and Müller 1981), *R. aconitifolius* s.l. or *R. glacialis* (see Huber 1988) as the other parent, not a single hybrid could be obtained.

The taxa of the *R. alpestris*-group have distinct areas of geographic distribution. Zones of overlap are rare; only the eastern Alps and the southern Carpathians host more than a single species. In both cases, these are the calcicole *R. alpestris* and the calcifuge *R. crenatus*. In the Carpathians, the two taxa occur on separate mountains. In contrast, in the eastern Alps (e.g. on the Planneralm in Austria), their members grow in close proximity, though in different habitats, with *R. alpestris* occurring on ledges of limestone rock and *R. crenatus* in calcifuge snowbeds. Despite the neighborhood, no intermediate plant has been found and no introgression between the respective microsites can be inferred from the morphology of corresponding individuals. As observed during our visits (in 1976, 1978, and 1992), *R. alpestris* flowers earlier in the season than *R. crenatus*, which is covered by snow for a longer time. The difference in flowering time hampers cross-pollination, but the latter is not entirely precluded, because partial overlap in flowering time is possible. Even if cross-pollination happens, hybrids can hardly be expected. Results of crossing experiments between *R. alpestris* and *R. crenatus* showed reduced fruitset (Müller and Baltisberger 1984). Very few of the produced fruits can germinate, and the hybrids show strongly reduced fertility. Although the taxa in the *R. alpestris*-group share the same ploidy level and karyotype, they are more or less well isolated reproductively, either by distinct geographic distribution areas or by reduced compatibility.

Final comments

Analysis of karyotypes in the *Ranunculus alpestris*-group partly confirms relationships inferred from molecular phylogenies, and yields important information, even though chromosome numbers and ploidy levels are identical among the taxa. It would be interesting to further correlate karyotypes and molecular data to test for their congruence across the entire genus.

According to the published phylogenies of *Ranunculus* (Johansson 1998; Hörandl et al. 2005; Paun et al. 2005) the ‘white-flowered European alpiners’ are situated on rather basal branches of the respective trees. All these taxa show the same karyotype. It is interesting that the alpine yellow-flowered *R. thora*-group, which in some phylogenies partly also occupies basal branches, shows a similar karyotype. The latter, therefore, probably represents a ‘primitive’ karyotype in the genus that has been maintained in the alpine zone.

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