Cytotaxonomical Studies in South Indian Ranunculaceae

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The Ranunculaceae consist of 35 genera and perhaps 1500 species, chiefly distributed in the cooler temperate regions of the earth and especially of the Northern hemisphere (Lawrence 1966). Roy and Sharma (1971) have cytologically studied 15 species of *Anemone*, *Aquilegia*, *Caltha*, *Clematis* and *Ranunculus* from Western and Eastern Himalayas. In South India, the taxa of Ranunculaceae are thriving only at higher altitudes of Western Ghats, particularly during the rainy seasons. There are 5 wild genera, namely *Naravelia*, *Clematis*, *Ranunculus*, *Thalictrum* and *Anemone*, consisting of 16 species in South India (Gamble 1956). Besides, the species of *Delphinium*, *Anemone*, *Nigella*, *Aconitum*, *Aquilegia* and rarely *Ranunculus* and *Eranthis* are ornamentally grown in Ooty, Kodaikanal and Yercaud of Western Ghats. Most of the wild species of South Indian Ranunculaceae have not been cytologically investigated so far. Therefore, the present investigation has been undertaken to understand the cytological relationship among the species of this taxonomically interesting and ornamentally important family.

Materials and methods

The wild species studied were collected from various localities of Ooty and Kodaikanal of Western Ghats (Table 1). The seeds of cultivated species namely, *Aquilegia canadensis*, *Nigella damascena*, *Delphinium ajacis*, *D. belladonna* and *Anemone japonica* were obtained from the Botanic Garden, Ooty. The seeds were sown in flat pots in our Botanic Garden, Annamalai University and the root tips were obtained from the seedlings for mitotic studies. The seedlings of the wild species were grown in our Botanic Garden in 1 foot clay pots under shade during rainy season when the climatic condition is favourable. Young and healthy root tips were obtained from the seedlings for mitotic studies.

For comparison of karyotypes of all the species, root tips were pretreated in saturated solution of 0.02% hydroxyquinoline kept at 4°C for 3 hours. Fixative used was acetic ethanol (1:3) for 3 hours. Root tip squashes were made following the schedule of iron alum haematoxylin squash technique described by Marimuthu and Subramaniam (1960). Camera lucida drawing was done at a magnification of $\times 1250$ and some of the mitotic stages were photographed.

Observations

The particulars regarding the species studied, the present reports of chromosome

Figs. 1-22. 1, *Naravelia zeylanica*, metaphase $2n=16$. 2 and 3, *Clematis wightianus*, metaphases, $2n=16$ and 32 respectively. 4-5, *Ranunculus wallichianus*, prophase and metaphase, $2n=32$; 6 to 8, *R. muricatus*. 6, metaphase, $2n=40$, 7 and 8, anaphasic and telophasic bridges. 9 to 11: *R. subpinnatus*. 9, metaphase, $2n=32$. 10 and 11, anaphasic bridges. 12 and 13, *R. reniformis* metaphase, $2n=46$ and 56 respectively. 14 and 15, *Thalictum javanicum*, prophase and metaphase, $2n=16$. 16, *T. dalzellii* metaphase, $2n=16$. 17 and 18, *T. saniculaeforme*, metaphases, $2n=16$ and 32 respectively. 19, *Aquilegia canadensis*, metaphase, $2n=32$. 20 and 21, *Nigella damascena* metaphases, $2n=12$ and 14 respectively. 22 to 24=*Delphinium belladonna*, 22, metaphases, $2n=24$. (Figs. 23, 24: see other page figures).

numbers, previous reports of chromosome numbers, authors and years are furnished in Table 1.

In *Anemone rivularis* there are $2n=24$ chromosomes. Besides, there are rarely tetraploid cells with 48 chromosomes. According to Roy and Sharma (1971), the population of *A. rivularis* collected from Shillong had $2n=16$ chromosomes and the population of this species collected from Darjeeling consisted of $2n=28$ chromosomes. In *Ranunculus subpinnatus*, there are rare occurrences of anaphasic bridges

Table 1.

Sl. no.	Species studied	Present report of chromosome no. (2n)	Previous report Author and Year.
1	<i>Naravelia zeylanica</i>	16	16 Raghavan and Arora 1958
2	<i>Clematis wightiana</i>	16*	—
3	<i>Ranunculus wallichianus</i>	32*	—
4	<i>R. muricatus</i>	40	48 Langlet 1927a, Larter 1932, Gregory 1941
5	<i>R. subpinnatus</i>	32*	—
6	<i>R. reniformis</i>	46*	—
7	<i>Thalictrum javanicum</i>	16	42 Kuhn 1928a
8	<i>T. dalzellii</i>	16*	—
9	<i>T. saniculaeforme</i>	16*	—
10	<i>Aquilegia canadensis</i>	32	14 Winge 1925, Gregory 1941, Linnert 1961, Lewis <i>et al.</i> 1962
11	<i>Nigella damascena</i>	12	12 Langlet 1927a, Gregory 1941, Bhattacharyya 1958
12	<i>Delphinium ajacis</i>	16	16 Langlet 1927a, Gregory 1941, Basale Jain 1962
13	<i>D. belladonna</i>	24	48 Langlet 1927c
14	<i>Anemone rivularis</i>	24	16 Langlet 1932, Gregory 1941
15	<i>A. japonica</i>	24	16 Heimbürger 1959, 16 Langlet 1932, Gregory 1941

* First record of chromosome numbers.

(Figs. 10, 11) and precocious movements of chromosomes at metaphase. Together with diploid cells ($2n=46$) there are rarely aneuploid cells with 56 somatic chromosomes in *R. reniformis*. Similarly, together with diploid cells ($2n=40$), there are rarely aneuploid cells with 56 chromosomes in *R. muricatus*. Besides, there are rare occurrences of anaphasic and telophasic bridges in this species (Figs. 7, 8). In *R. wallichianus*, there are chromosomal variabilities such as $2n=32$ (54%), $2n=36$ (25%) and $2n=42$ (21%) chromosomes, of which $2n=32$ is the actual diploid number of this species. In *Nigella damascena* there are cells with $2n=12$ (65%) and $2n=14$ (35%) chromosomes, of which $2n=12$ is the actual diploid chromosome number of this species. In *Aquilegia canadensis*, there are cells with $2n=30$ (35%) and $2n=32$ (65%) chromosomes, in which $2n=32$ is the diploid chromosome number of this species.

The chromosomes of Ranunculaceae show considerable variations in size from the largest to shorter but they are mostly large, medium and short. It is of particular interest to note that the shorter chromosomes measured more than a millimicron, while the longest chromosome measures more than 10 millimicrons. Based on the length, the chromosomes have been grouped into shorter, short, medium sized long, longer and the longest chromosomes. Under each of these groups, the following categories of chromosomes have been recognized.

Figs. 23–29. 23 and 24: *Delphinium belladonna*. 23, metaphase, $2n=32$. 24, prophase. 25, *D. ajacis* metaphase, $2n=16$. 26 and 27, *Anemone rivularis*, metaphases, $2n=24$ and 48 respectively. 28 and 29, *A. japonica*, metaphases, $2n=24$ and 34 respectively.

Longest (more than $12.5 \mu\text{m}$)

- Type A: chromosome with submedian primary centromere and subterminal secondary centromere.
- Type B: chromosome with submedian centromere.
- Type C: chromosome with median centromere.

Longer (10.1 to $12.5 \mu\text{m}$)

- Type D: chromosome with submedian primary and subterminal secondary centromeres.
- Type E: chromosome with submedian centromere.
- Type F: chromosome with median centromere.
- Long (7.6 to 10.0 μm)
- Type G: chromosome with submedian primary and subterminal secondary centromeres.
- Type H: chromosome with submedian centromere.
- Type I: chromosome with median centromere.
- Type J: chromosome with subterminal centromere.
- Medium (5.1 to 7.5 μm)
- Type K: chromosome with submedian primary centromere and subterminal secondary centromere.
- Type L: chromosome with two subterminal centromeres, one on either end.
- Type M: chromosome with submedian centromere.
- Type N: chromosome with median centromere.
- Type O: chromosome with subterminal centromere.

Figs. 30–32. 30, *Ranunculus reniformis*, $2n=46$. 31, *Delphinium belladonna*, $2n=32$. 32, *Nigella damascena*, $2n=12$.

Short (2.6 to 1.0 μm)

- Type P: chromosome with submedian primary centromere and subterminal secondary centromere.
- Type Q: chromosome with two subterminal centromeres, one on either end.
- Type R: chromosome with submedian centromere.
- Type S: chromosome with median centromere.

Type T: chromosome with subterminal centromere.

Shorter (1.0 to 2.5 μm)

Type U: chromosome with median centromere.

Type V: chromosome with subterminal centromere.

The total number of satellited chromosomes has been found to vary from species to species. The karyomorphological features of the various species studied are summarised in the following table.

Table 2. Summarised karyomorphological features of the taxa investigated here

Taxa	1	2	3	4	5	6	7	8
<i>Naravelia zeylanica</i>	16	6.0–10.0	4	4	2	6	128.0	8.00
<i>Clematis wightianus</i>	16	4.5–10.0	2	2	4	8	100.0	6.25
<i>Ranunculus wallichianus</i>	32	3.5– 7.0	6	6	8	12	159.0	4.96
<i>R. muricatus</i>	40	2.0– 4.0	4	10	12	14	115.0	2.87
<i>R. subpinnatus</i>	32	4.0– 8.0	4	8	10	10	171.0	5.34
<i>R. reniformis</i>	46	2.0– 4.2	2	16	14	14	143.2	3.11
<i>Thalictrum javanicum</i>	16	1.5– 3.8	2	2	6	6	34.6	2.16
<i>T. dalzellii</i>	16	1.3– 4.0	2	4	6	6	36.6	2.28
<i>T. sanicelaeforme</i>	16	1.4– 3.0	2	2	4	8	31.2	1.95
<i>Aquilegia canadensis</i>	32	4.5– 7.0	4	8	8	12	171.0	5.34
<i>Nigella damasena</i>	12	8.0–16.0	2	2	6	2	144.0	12.9
<i>Delphinium ajacis</i>	16	2.0– 4.0	2	4	4	6	46.0	2.87
<i>D. belladonna</i>	24	4.0–10.0	2	6	10	6	154.0	6.42
<i>Anemone rivularis</i>	24	3.5– 9.0	2	6	4	12	135.0	5.62
<i>A. japonica</i>	24	3.5–11.5	2	4	6	12	162.0	6.75

The descriptions of the various columns of Table 2 are as follows:

- 1 Chromosome number (2n).
- 2 Size range in millimicrons.
- 3 Number of chromosomes with secondary constrictions.
- 4 Number of chromosomes with submedian centromere.
- 5 Number of chromosomes with median centromere.
- 6 Number of chromosomes with subterminal centromere.
- 7 Absolute chromosome length.
- 8 Average chromosome length.

Discussion

As early as 1939, Coonen suggested that $n=8$ chromosomes have originated from $n=7$ by deficiency, whereas Kurita (1955a) considered $n=8$ with long 'chromosomes to be primitive and $n=7$ to be advanced in evolution. He suggested (1955b) that $n=8$ series can be classified into two subseries according to chromosome size. First record of chromosome number has been made in *Ranunculus wallichianus* ($n=32$), *R. subpinnatus* ($2n=32$) and *R. reniformis* ($2n=46$). All these species are high polyploids as revealed by the present study and the former two species are euploids and the latter an aneuploid. In *R. muricatus*, the previous authors reported

varying chromosome numbers, such as, $2n=16$, 47 and 48 (Kurita 1955a, Langlet 1932, and $2n=32$ (Roy and Sharma 1971) as against the present observation of $2n=40$ chromosomes in this species. Perhaps, the population of *R. muricatus* herein studied may be a pentaploid, originated probably from $n=8$ series.

As evidenced by the present investigation, higher polyploidy plays an important role in speciation, as far as the South Indian species of *Ranunculus* are concerned.

In *Thalictrum*, the basic chromosome number may be $n=8$. In *T. javanicum*, Kuhn (1928a) (Vide Fedorov 1974), has reported $2n=42$ chromosomes, but the present study shows $2n=16$ chromosomes in this species. The diploid chromosome numbers have been reported for the first time for *T. dalzellii* ($2n=16$) and *T. saniculaeforme* ($2n=16$). Therefore, all the three species of *Thalictrum* studied here are diploids. Besides, the chromosomes are also smaller in size with mostly submetacentric and telocentric chromosomes.

Winge (1925), Gregory (1941), Linnert (1961) and Lewis *et al.* (1962) have reported $2n=14$ chromosomes in *Aquilegia canadensis*. Besides, Roy and Sharma (1971) have also reported $2n=14$ chromosomes in this species. But, the population of this species studied here shows $2n=32$ chromosomes. This may be a polyploid probably originated from $n=8$ series as against the $n=7$ series, as suggested by the previous authors.

The present report of chromosome number in *Nigella damascena* ($2n=12$) confirms the earlier record for this species (Langlet 1927a, Gregory 1941, Bhattacharya 1958b). This is a primitive species with mostly metacentric and longer chromosomes.

In *Delphinium ajacis*, the diploid chromosome number is $2n=16$ and this is in confirmation with the earlier observations (Table 1). Langlet (1927a) has reported $2n=48$ chromosomes in *D. belladonna* as against the present report of $2n=24$ chromosomes in this species. The basic chromosome number in *Delphinium* may be $n=8$ and *D. ajacis* is a diploid and *D. belladonna* is a polyploid. The rare occurrences of polyploid cells in these two species, that is $2n=32$ and the earlier observations in some of the species of *Delphinium* (Fedorov 1974), show that polyploidy plays an important role in speciation in this genus.

Cytological works carried out in the genus *Anemone* have so far shown that species are characterised by two series of chromosome numbers $n=7$ and $n=8$. Moffett (1932a) suggested that $n=7$ has originated from $n=8$ by fusion. Kurita (1957) observed that the basic karyotype of $n=7$ series has six large metacentric chromosomes and one short acrocentric one and that is ancestral to $n=8$. The basic karyo-type of $n=8$ series, on the other hand, has four metacentric, one submetacentric and three acrocentric chromosomes. Bocher (1959) studied the chromosome morphology and meiotic behaviour in *A. richardsoni* and inferred that $n=7$ has been derived from $n=8$ chromosomed ancestors. In both *A. rivularis* and *A. japonica* there are $2n=24$ chromosomes, indicating that they are polyploids derived from $n=8$ chromosome series. Therefore, it is clear that polyploidy plays important role in speciation in the genus *Anemone*.

In *Clematis*, unlike the other genera of Ranunculaceae, a remarkable uniformity in chromosome number has been reported all being multiples of $n=8$ (Kurita 1955a).

But, Roy and Sharma (1971) have shown that the diploid chromosome number was $2n=18$ in *C. flammula* from Eastern Himalayas and $2n=36$ in *C. paniculata*, indicating the diploid and ploid representatives of $n=9$ series respectively. But, the present investigation reveals the first record of chromosome number in *C. wightianus*, that is $2n=16$, again a diploid representative of the $n=8$ series. Further investigations will be taken up to study the chromosome number of some more species of *Clematis* of Western Ghats, in which no cytological studies have been made so far and final conclusion will be arrived at regarding the actual basic number of the species of *Clematis* of Western Ghats.

The present report of $2n=16$ chromosomes in *Naravelia zeylanica* confirms the earlier observations of chromosome number in this species (Raghavan and Arora 1958). The chromosomes of this species are larger in size. Both in the same diploid chromosome number and larger size of chromosomes, *Clematis wightianus* and *Naravelia zeylanica* are closely related together.

In general, the family shows a wide range of somatic chromosome numbers from $2n=12$ to $2n=46$, as revealed by the present investigation. The basic chromosome number may be $n=8$, as most of the taxa studied possess $2n=16$ chromosomes (6 species) or multiples of 8, that is $2n=24$ or $2n=32$ or $2n=40$ (7 species). Other diploid numbers should have arisen by decrease or increase of chromosomes from the basic number $n=8$, followed by polyploidization. Another interesting point that emerges from the present investigation is that Ranunculaceae consist of species that are highly variable and polyphyletic in nature. It is evidenced by the presence of wide range of somatic chromosome numbers, occurrence of wide range of chromosome sizes from the longest to shorter (Table 3) and the presence of considerable variations in total chromosome lengths of various species studied (Table 2).

Summary

Fifteen species of Ranunculaceae under eight genera have been studied, mostly collected from different regions of Ooty and Kodaikanal of Western Ghats. First record of chromosome numbers has been made in *Ranunculus wallichianus*, *R. subpinnatus*, *R. reniformis*, *Clematis wightianus*, *Thalictrum dalzellii* and *T. saniculaeforme*. On the basis of previous and present cytological data, there are $n=7$ and $n=8$ series in *Ranunculus*. All the species of *Ranunculus* studied here are higher polyploids and both aneuploidy and euploidy play important role in speciation.

The previous karyological analyses of *Anemone* reveal two distinct series, one with $n=8$ and the other $n=7$. Both *A. rivalaris* and *A. japonica* have $2n=24$ chromosomes and they should have derived from $n=8$ series. Both in the larger size and the same number of chromosomes, *Naravelia zeylanica* and *Clematis wightianus* are related together.

The presence of fewer number of chromosomes and larger size of them indicates that *Nigella damascena* is a primitive species. All the three species of *Thalictrum*, namely *T. javanicum*, *T. dalzellii* and *T. saniculaeforme* possess $2n=16$ chromosomes and perhaps, these taxa are diploids derived from $n=8$ series. The chromosomes are comparatively smaller in size in all these species.

Aquilegia canadensis has $2n=32$ chromosomes. This may be a polyploid and it should have originated from $n=8$ basic chromosome series.

The population of *Delphinium ajacis* studied here is a diploid and that of *D. belladonna* a triploid and the basic chromosome number in *Delphinium* may be $n=8$. As far as this genus is concerned, higher polyploidy plays important role in specification.

In general, the taxa of Ranunculaceae studied show a wide range of chromosome numbers from $2n=12$ to $2n=46$. As most of the taxa studied show either $2n=16$ or multiples of $n=8$, the basic number of the family may be $n=8$ and the other basic numbers should have derived from it. The species are highly variable and polyphyletic in nature, as evidenced by the wide range of chromosome numbers, chromosome sizes and total chromosome lengths of various species studied.

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