CYTOLOGICAL STUDIES ON INDIAN REPRESENTATIVES OF THE GENUS *VIOLA*

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INTRODUCTION

The genus *Viola* is widely distributed in the Himalayas both in the Eastern and Western zones, ranging from subtemperate to even alpine zones. A large number of phenotypic variations has been observed to occur within the same species. Moreover, one species, *V. tricolor* (Pansy) is widely cultivated for its large number of horticultural forms.

The chromosome studies on this genus growing in Europe and North America have yielded very interesting cytological data—helpful in working out its taxonomy and distributional ecology (Clausen, 1964, 1967; Harvey, 1966; Löve and Löve, 1956; Sokolovskaya, 1963, 1965). A large number of chromosome series have been found and in certain cases members of a distinct subsection are seen have a sympatric distribution. On the other hand, there are certain groups in which species of a subsection and even subspecies of a species occupy distinct areas in ecologically diverse regions (Clausen, 1964). Such contrasting characters within a genus are quite striking. Clausen (1967) has, however, suggested that in the genus *Viola*, the 6-chromosome series is prevalent in section Chamaemelanium, the 12-chromosome series in section Plagiostigma, the 10-chromosome series in section Rostellatae, whereas variable numbers are seen in section Melanium even though 6 and 10 are more prevalent (Valentine, 1962; Clausen, 1964) than others.

Though so much interesting work has been done, cytological work on representatives from India is lacking. The genus not only has a wide distribution in Himalayas, but a large number of biotypes are also found, in addition to its cultivated forms. In view of these facts, taken in conjunction with the lack of cytological data on Indian representatives, the present investigations were undertaken. It was hoped that a thorough investigation on Indian species may reveal inteesting data from cytotaxonomical and cytoecological stand-points as yielded by the European and North American representatives.

MATERIALS AND METHODS

A list of the specimens of *Viola* studied along with their voucher numbers and places of collection with altitudes is given below:

No.	Name of the plant	Voucher No.	Place and altitude of collection
1.	*Viola biflora* Linn. (Type I, with yellow flowers)	A.S. 2172	Valley of flowers, Western Himalayas, 13,000 feet
2.	*V. biflora* Linn. (Type II, with violet flowers)	A.S. 1165	Darjeeling, Eastern Himalayas, 6,000 feet
3.	*V. distans* Wall.	A.S. 2	Darjeeling, E. Himalayas, 6,500 feet
4.	*V. hookeri* Thoms. ex Hook. f. (Type I, with mauve flower)	A.S. 77	Tonglu, E. Himalayas, 10,500 feet
5.	*V. hookeri* Thomas. ex Hook. f. (Type II, with white flower)	A.S. 875	Ghoom-Bhanjang forest, E. Himalayas, 6,000 feet
6.	*V. odorata* Linn. white with blue on the petals)	A.S. 891	Darjeeling, E. Himalayas, 6,000 feet
7.	*V. serpens* Wall. Type I, flowers streaks	A.S. 892	Darjeeling E, Himalayas 6,000 feet
8.	*V. serpens* Wall. (Type II, with violet flower)	A.K.S. 929	Near Hemkund, W. Himalayas 14,000 feet
9.	*V. tricolor* Linn. (Var I, petals violet and yellow with velvety stripes on the inner surface)	A.K.S. 1193	Calcutta, (Sea level)
10.	*V. tricolor* Linn. (Var II, petals yellowish-white with violet stripes)	A.K.S. 1194	Calcutta, (Sea level)

The plants were identified through the courtesy of the authorities at the National Herbarium, Shibpore.

For the study of somatic chromosomes, excised root tips were pretreated in different chemical agents for different periods and with different concentrations (*vide* Sharma and Sharma, 1965). The best results were obtained with a treatment in saturated aqueous solution of aesculin for five minutes at a temperature of 1 to 2° C and subsequently at 10 to 12° C for seventy minutes (Sharma and Sarkar, 1955). They were then rinsed in water, fixed in a mixture of glacial acetic acid and absolute ethyl alcohol (1:2) for one hour, treated in 45% acetic acid for fifteen minutes and stained by heating in a mixture of 2% acetic-orcein solution and normal hydrochloric acid (9:1) for a few seconds, keeping for overnight and finally squashing in 45% acetic acid.

For meiotic studies, flower buds of suitable size were fixed in glacial acetic acid and absolute ethyl alcohol mixture (1:2) overnight, stored in 70% ethyl alcohol and later smeared in 1% aceto-carmine solution with the help of a scalpel. Treatment in 45% acetic acid for ten minutes before smearing gave better results.

Figures were drawn always from the temporary preparations at a table magnification of approximately × 2,400, using a Zeiss compensating eye-piece of × 12·5 and a Zeiss 1·3 apochromatic objective with an aplanatic condenser. In the figures the chromosomes with secondary constrictions are drawn in outline only.

OBSERVATIONS

The different species and varieties of the genus *Viola* so far investigated show a wide range of chromosome numbers from as low as $n = 6$ to as high as $n = 36$ with a considerable degree of structural alterations of the chromosomes. Even the same species collected from different areas and altitudes shows a wide variation both in number and structure of chromosomes.

Karyotypes of all the species and varieties investigated in the present paper reveal that on the basis of gross morphological features, a number of chromosomal types is common to all of them. A critical analysis, however, shows that the different species and varieties differ in different combinations of these types as well as in the finer morphological details which may be considered as criteria for identification of these taxa. So it will be convenient to describe the general types separately at the beginning and their finer differences in the description of the karyotype for each species and variety. Though the chromosomes are in general short, on the basis of their relative length they can be divided into three main groups, viz., comparatively long, medium and short.

A general description of the different types, depending on the locations of constrictions and size of the chromosomes is given below:

Type A : Comparatively long chromosomes, each with a nearly median primary constriction and a satellite attached to the distal end of the shorter arm.

Type A : Very similar to type A, with the difference that the satellite in this case is not split and attached to one side of the chromosome (i.e., one-sided).

Type B : This includes a series of long to short chromosomes, with median to nearly median primary constrictions.

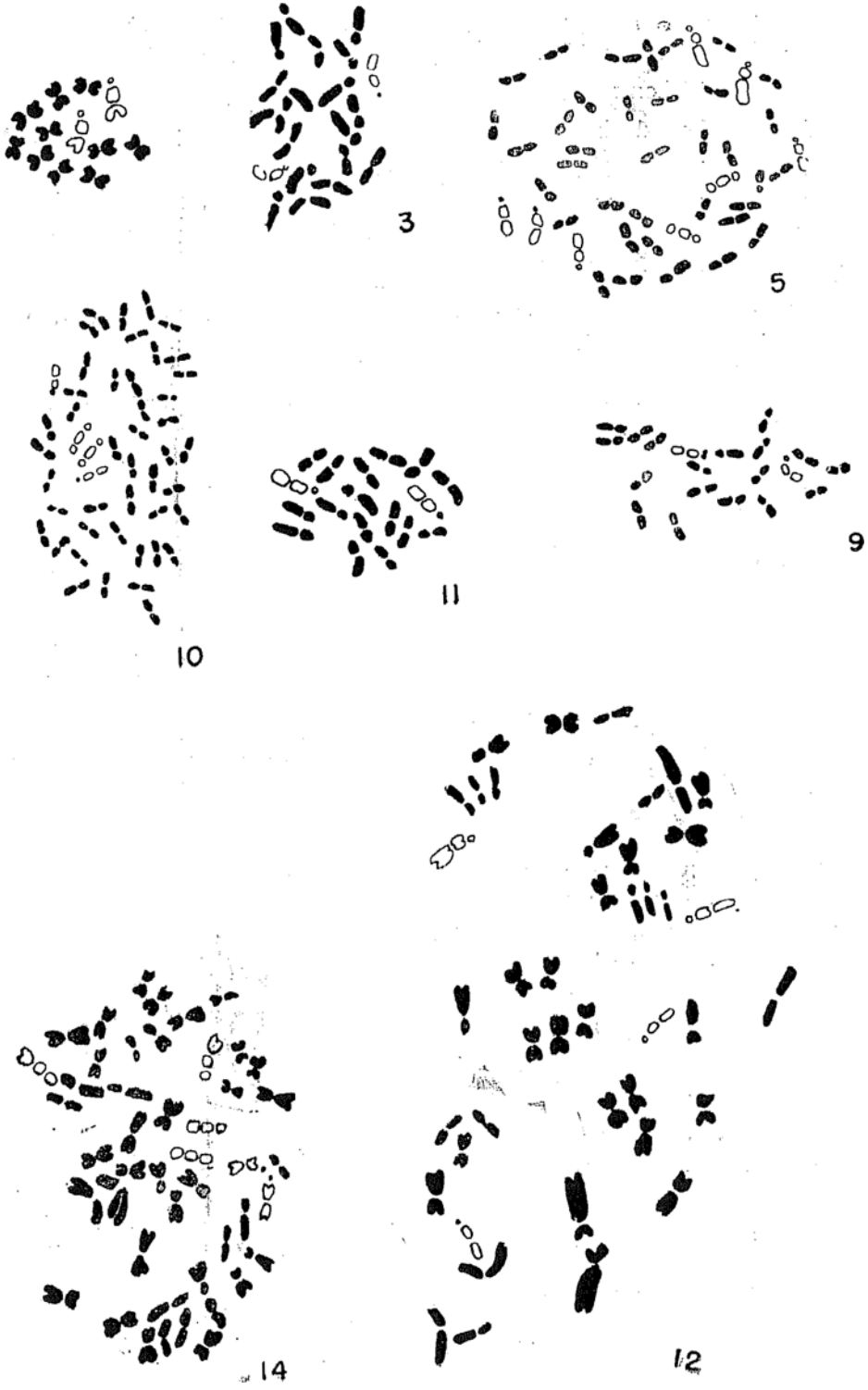
Type C : Graded long to short chromosomes, with submedian to nearly submedian primary constrictions.

Type D : Long chromosome, each with two constrictions, primary and secondary, one nearly median and the other nearly submedian in position.

Type E : Long chromosomes, each with two distinct constrictions, primary and secondary, both submedian in position. The end segments of the chromosome are almost equal in length and the middle segment is longer than the distal arms.

Type E′ : Similar to type E, but the three arms are nearly equal.
Meiosis is observed to be regular.

1. *Viola biflora* Linn., *Type* 1. $2n = 12 = A_2 + B_8 + C_2$; $2{\cdot}5\,\mu$ to $1{\cdot}5\,\mu$ (Figs. 1 and 1 a); $n = 6$ bivalents in diakinesis (Fig. 2).
2. *Viola biflora* Linn., Type II. $2n = 20 = A_2 + B_8 + C_{10}$; $2{\cdot}7\,\mu$ to $1{\cdot}9\,\mu$ (Figs. 3 and 3 a); $n = 10$ bivalents in diakinesis (Fig. 4).


3
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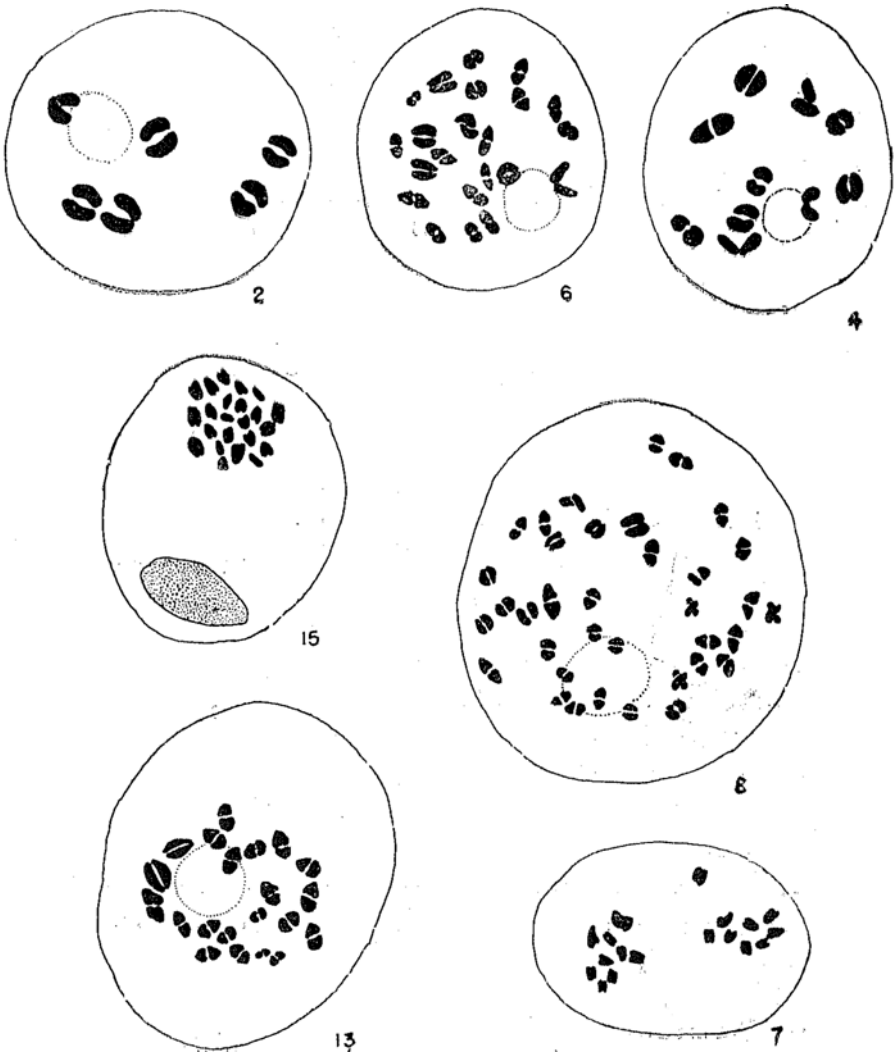
3. *V. distans* Wall. $2n = 44 = A_6 + B_{36} + E_2$; 2·6 μ to 1·2 μ (Figs. 5 and 5 a); $n = 22$ bivalents (F g. 6).
4. *V. hookeri* Thoms. ex Hook.f., Type I. $n = 9$.

This plant showed nine chromosomes in each pole in Metaphase II (Fig. 7).

5. *V. hookeri* Thoms. ex Hook.f., Type II. $n = 36$

This plant showed thirty-six bivalents in diakinesis (Fig. 8) with normal meiotic behaviour.

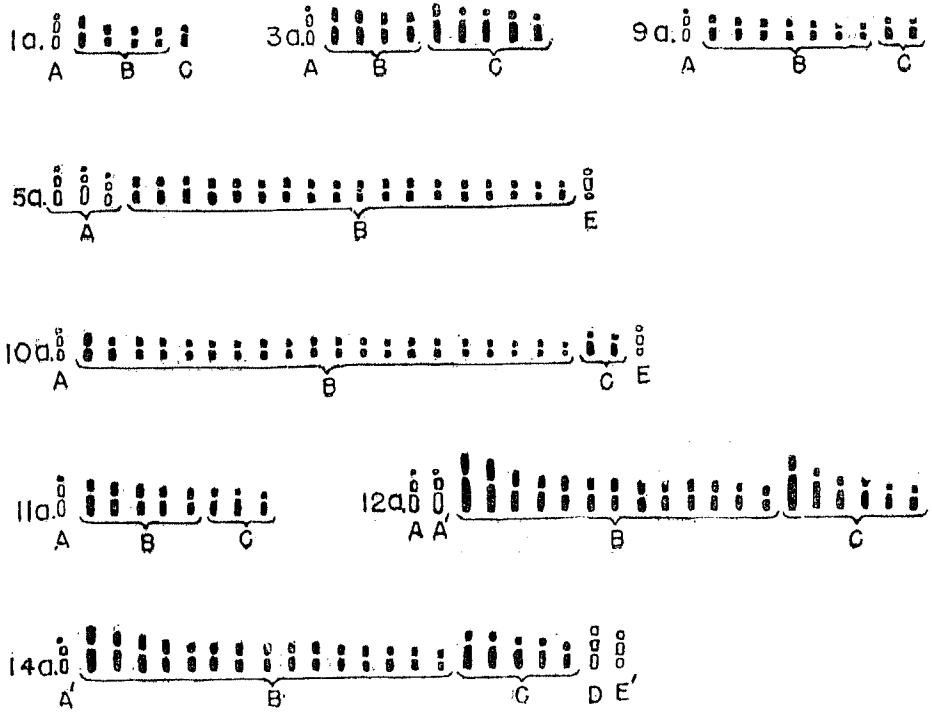
6. *V. odorata* Linn. $2n = 20 = A_2 + B_{14} + C_4$; 2$\mu$ to 1·2 μ (Figs. 9 and 9 a).
7. *V. serpens* Wall. Type I. $2n = 48 = A_2 + B_{40} + C_4 + E_2$; 2·1 μ to 0·85 μ (Figs. 10 and 10 a).



8. *V. serpens* Wall. Type II $2n = 18 = A_2 + B_{10} + C_6$; 2·7 μ to 1·6 μ (Figs. 11 and 11 a).

9. *V. tricolor* Linn. Var. I. $2n = 42 = A_2 + A_2' + B_{26} + C_{12}$; (Figs. 12 and 12 a); $n = 21$ bivalents (Fig. 13).

10. *V. tricolor* Linn. Var. II. $2n = 46 = A_2' + B_{30} + C_{10} + D_2 + E_2'$; 3·1 μ to 1·4 μ (Figs. 14 and 14 a); n=13 chromosomes in metaphase II (Fig. 15).



Discussion

The genus *Viola* has been classified under different sections and sub-sections by De Candolle (1823) and Engler and Prantl (1915), whereas Hutchinson (1959) did not classify as such. Of the species investigated here *V. biflora* falls under the section Dischidium (sub-section Brevicalcaratae), *V. odorata* and *V. tricolor* under section Erpetion, *V. serpens* under section Nomimium (sub-section Serpentes) in Engler and Prantl's system of classification. *V. hookeri* and *V. distans* could not be traced in Engler's system. The section Erpetion was originally included under the section Melanium of De Candolle.

It has already been pointed out by Clausen (1964) that in section Melanium, chromosome number is variable having tendencies towards 6 and 10 series, interspersed with aneuploids. In the present investigation too, only one species belonging under this section of De Candolle shows variability, viz., *V. tricolor* with $2n = 42$ and 46 chromosomes. However, *V. tricolor*, though included under Melanium section by De Candolle, falls under Erpetion of Engler and Prantl together with *V. odorata* ($2n = 20$). Even though the variability in chromosome number, that is, a difference between 6 and 10 series is not taken as a criterion for categorization in view of intra-specific variations, the chromosome morphology of the two species does not justify their inclusion within the same section. The chromosome size of *V. odorata*, in a complement showing chromosome numbers as low as $2n = 20$, is much shorter than that even in polyploid individuals of *V. tricolor* having $2n = 42$ and 46 chromosomes. Moreover, certain chromosomes of *V. tricolor* are unusually longer than the chromosomes of *V. odorata* (vide histogram and idiogram). Such a difference in chromosome size is difficult to explain, specially in view of the very small size in diploids. It is therefore preferable to separate *V. odorata* from section Erpetion and include it under some other section in which not only the chromosome morphology but other evidences too point to its affinity. If it is retained within Erpetion (Engler and Prantl, 1915) it is implied that the section does not represent a natural assemblage and indicates heterogeneous grouping. It may be mentioned here that in De Candolle's system, *V. odorata* and *V. tricolor* fall under two sections, the former under Nomimium and the latter under Melanium.

The two cytotypes of *V. serpens* studied show $2n = 18$ and 48 chromosomes. $2n = 18$ may represent a series of 6 or 9 whereas $2n = 48$ suggests a series of 6. As meiosis in the former could not be studied it is difficult to suggest a triploid constitution. The karyotype study, however, has revealed the presence of chromosome pairs which might have been due to structural alteration of chromosomes playing a role in evolution. Such structural alterations might have resulted in apparently similar chromosome morphology so that the chromosomes could be grouped in mitosis. However, detailed meiotic data are essential to substantiate its triploid constitution. Pending such an investigation the presence of two different chromosome series, one of 6 and the other of 9, may be hypothesised in *V. serpens*. Such intraspecific variations suggest that the two series are quite

related and one is derived from the other. Their relationship is also marked in their karyotypes where considerable similarity in chromosome morphology is noted. The presence of slightly longer chromosomes in low chromosome numbered complement and comparatively shorter chromosomes in the high chromosome numbered species indicates the importance of steady diminution of chromosome size along with polyploids in evolution. Existence of distinctly different chromosome series in the same species has so far not been reported in *Viola*, excepting in *V. kitaibeliana*, though aneuploids are often found. Similar presence of two distinct chromosome series $2n = 12$ and 20 in two populations has been observed in *V. biflora* of the section Dischidium. The chromosome morphology too, in the two populations, shows marked similarity. This is also another example of the presence of two distinct chromosome series at an intraspecific level indicating their relationship.

Therefore, the present investigation on *V. serpens* and *V. biflora* has for the first time yielded data suggesting that numerical series of chromosomes should not play an important role in categorizing species assemblages.

In *V. hookeri* clear polyploid cytotypes have been recorded showing $n = 9$ and $n = 36$ chromosomes, whereas in *V. distans* $2n = 44$ chromosomes have been observed. So far as chromosome morphology is concerned, *V. distans* shows remarkable similarity with *V. serpens*.

The species of *Viola* investigated here and in previous publications indicate in their cytology certain features which are of fundamental cytoecological importance (Baker, 1953, 1957; Beuzenberg and Hair, 1959; Börgmann, 1964; Bruun, 1932; Carr, 1969; Chuang et al., 1963; Clausen, 1929, 1931, 1964; Clausen et al., 1940; Fothergill, 1944; Gadella, 1963; Gadella and Kliphuis, 1963; Gershoy, 1934; Griesinger, 1937; Harvey, 1966; Jörgensen et al., 1958; Kondo et al., 1956; Larsen, 1954; Lewis et al., 1962; Löve and Löve, 1953, 1966; Miyaji, 1929, 1930; Moore, 1963; Packer, 1964; Pettet, 1964; Schmidt, 1961, 1962, 1963, 1964 a, b; Skalinska and Pogan, 1959, 1966; Sköttsberg, 1955; Sokolovskaya, 1963, 1965, 1966; Sokolovskaya and Strelkova, 1960, 1962; Sorsa, 1963; Taylor and Brockman, 1966; Valentine, 1949, 1962). In most of the species studied, e.g., *V. serpens*, *V. hookeri* and *V. biflora*, where different cytotypes have been recorded, diploids inhabit regions of higher altitude as compared to their polyploid counterpart. The occurrence of diploids in higher altitude of Himalayas gives an indication of their path of migration from Europe and Mediterranean zones where *Violas* grow in abundance. A detailed investigation in this aspect is desirable. In any case, the genus *Viola* represents an unusual example in which extreme climatic conditions are tolerated by diploids whereas the polyploids abound in comparatively moderate climate (Cain, 1944; Löve and Löve, 1953).

SUMMARY

The present investigations on the chromosome study of ten Indian representatives of *Viola* have revealed interesting data from cytotaxonomical and cytoecological standpoints. The existence of two distinctly different chromosome series in the

same species, rather uncommon for this genus, has been recorded in *V. serpens* ($2n = 18$ and 48) and *V. biflora* ($2n = 12$ and 20). Such intraspecific variations imply that differences in numerical series of chromosomes should not necessarily play an important role in categorizing species assemblages. They have also been noted in *V. tricolor* ($2n = 42$ and 46) and polyploid cytotypes have been recorded in *V. hookeri* ($n = 9$ and 36). On the basis of chromosome data, it has been stated that the retention of *V. odorata* and *V. tricolor* under the section Erpetion as followed by Engler and Prantl, is not desirable. De Candolle in his system kept the two genera under two separate sections. Remarkable similarity between *V. serpens* ($2n = 18$ and 48) and *V. distans* ($2n = 44$) in chromosome morphology has been shown.

From a study of the chromosomes of different ecotypes it has been shown that in *Viola* extreme climatic conditions are tolerated by diploids, whereas the polyploids abound in comparatively moderate climate, which is rather uncommon for other genera.

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