DISCUSSION

Morphological considerations:

In the previous chapter of observations, the descriptive description of immediate concern for individual species is made. However, in this chapter other important points are discussed.


Following is the key given by them to separate the above three genera:

An artificial key to the 3 genera

1. Flowers under 3 cm across; stigmatic surfaces not flat and separate;

2. Ovary and capsule more or less erect, and parallel to the peduncle, not spreading at an angle to it; stigmatic surfaces in the form of small swellings on the edge of the lip. ......Peristylus
2. Ovary and capsule widely spreading at an angle
to the peduncle; stigmatic lobes standing
out as stalked appendages..........Habeneria

1. Flowers about 7.5 cm across; stigmatic surfaces
flat, almost confluent..................Platanthera

The present studies also give support to this generic
delimitation (Pl. I, Figs. 1,2,3). Key to identify the
eight species of Peristylus is already given in the observ­
ations.

Further, after careful observations of lip characters
it is realised in this study that a considerable diversity
exists in the species of the genus Peristylus Bl. Adams
(1959) and Dressler and Dodson (1960) are of the opinion
that primitive lip is more like a petal while the advanced
lip is variously modified and unlike petal. Thus, in the
present study considering lip as a taxonomic character in
speciation, the simple petal like lip without any lobation
observed in P.platangiaea perhaps has given rise to the
modified and complex lips of P.xanthochlorus on one hand
and P.ernestin on the other. In P.elatus and P.goodyeroides,
the lobation is found to be quite distinct. This tendency
of lobation increases in P.lawii where the side lobes are
slightly longer than midlobe and curved on the outer
margins. The midlobe tapers slightly and is truncate with a broad sinus. When the lip of *P. lawii* is compared with *P. stocksi*, the latter is found with longer side lobes which reveals its advance status. The sinus in *P. stocksi* is deep and broader whereas in *P. densus* and *P. xanthochlorus* it is shallow and the broadest; the midlobes of the three do not exhibit any marked structural differences. However, in *P. densus* the extreme distal portion of the side lobes is found to be thread like that spread apart and get curved upward and backward. From the above modifications of the lobes in the lip, *P. densus* and *P. xanthochlorus* attain an advanced position over *P. stocksi*.

Lip of *P. aristatus* occupy the same level of complexity as that of *P. xanthochlorus*. The side lobes in *P. aristatus* are long, linear and hanging but slightly curved upward. Midlobe is ligulate with obtuse and inflated apex and half the size of side lobes. The progressive modification leading to the complexity of lip character in all the eight species has been tabulated (Table 24). Thus, based on the morphological observations of the lip the evolutionary trends among the eight species of the genus Peristylus ill. may be graphically represented (Pl.XXI, Fig.1) in the chronology of their primitive to advanced status as:
Fig I  A tentative phylogenetic tree in the genus *Peristylus* BI
Looking to the habitat, habit, arrangement of leaves, their colour and texture, dense and lax nature of inflorescence, spur character etc., the species of *Peristylus* fall into 2 groups. The first group includes *P. plantagineus*, *P. elatus*, *P. goodyeroides*, and *P. lawn* which show primitive characters. They are usually found as an undergrowth in semi-evergreen forest, protected from biotic disturbances, where they maintain their steady growth. Being developed in shade, they are generally tall with their leaves fleshy, bottle green to brownish, few and confined in the middle of the stem. The inflorescence is compact, long and many flowered. Spur is sessile or scrotiform and shorter than the lip.

The second group consists of *P. stocksii*, *P. densus* and *P. xanthochlorus* which are found exposed to biotic stresses and in turn show reduced height. The leaves are thin, green and spirally arranged in the 2/3 lower portion of the stem. Inflorescence is lax with reduced number.
of flowers - an ease to obtain the pollinating agent. Spur is found to be saccate and as long as lip. These characters show advanced nature of the above three species. However, the case of *P. xanthochlorus* is more interesting. The plants are much reduced in height with rigid nature of stem and leaves. This seems to be a further adaptation to withstand the extreme stresses of environmental and biotic factors. *P. aristatus* shows mixture of primitive and advanced characters though with reference to lip character it attains a status equal to *P. xanthochlorus*. In spite of thriving in shady places of cut forest the plants are small in height. Leaves are thin, papery with rosette arrangement which are primitive characters. Spur in this species is sub-saccate and shorter than lip. The inflorescence is a lax raceme and flowers are few and distant as found in *P. xanthochlorus* - a feature of advancement.

Status of the four taxa in the complex of *H. heyneana* Landl., *H. subpubens* A.Rich., *H. cerea* Blatt. & McC. and *H. cerea* var. polyanthra Blatt. & McC. has already been discussed (in observation). *H. subpubens* seems to be a simple case of biotype of *H. heyneana* on morphological considerations. No constant characters were observed to keep *H. subpubens* as a separate species. Position of *H. cerea* as an independent species is quite clear by virtue of its distinct morphological
characters and allopatric distribution. However, after examining the plants of *H. cerea* var. *polyantha* in their type locality (3rd Tableland, Panchagani), it is concluded that these plants do not deserve separate varietal rank.

*H. longicarniculata* Graham is a clear and good species.

*Platanthera susannae* (L.) Lindl. is also a clear and good species.

**Cytological consideration:**

It has been long since realised that chromosomes in a plant are important to taxonomy in connection with their numbers, size and shapes. Such chromosome characters have been utilized as classificatory criteria in addition to the morphological features.

In the family Orchidaceae, there has been considerable cytological work. Miduno (1940b) was of the opinion that aneuploidy is a characteristic feature of this family. According to Duncan (cf. Withner, 1959), the extent of chromosome number variation among genera in a group may be as extensive as it is among the species within the genus. Further, he adds that speciation among Orchids may be due
to chromosome number variation accompanied by aneuploidy or euploidy as in case of *Paphiopedilum*, *Goodyera*, etc. or may be caused by the number of supernumeraries as in *Cypripedium aculeae*. Tanaka (1965) reported the result of chromosome counts in 111 species of Orchids and speculated that reduction of chromosome number might have played an important role in speciation. Jones (1967) reported the chromosome number of 123 species of Vanda and emphasized the importance of chromosome number in Orchid taxonomy. An exhaustive information on chromosomes in different groups of Orchids is reported by Kaminoto and his associates (l.c.).

So far the chromosome number reports on the members of genera *Habenaria* Willd., *Platanthera* L.C.Rich. and *Peristylus* Bl. are very few compared to the number of species in each genus.

(1) Chromosome number and Basic number

The basic (or base) number is defined as the primitive or original number from which polyploid numbers have been derived (Swanson, 1958). In practice the basic numbers of a genus are usually calculated from the lowest diploid numbers known in the group (Love and Love, 1961). In some cases the diploids may no longer exist or are not recognized
as such and only apparently polyploid numbers are reported within the genera concerned. A theoretical basic number has to be inferred in such cases.

Habenaria Willd.

The genus Habenaria is comprised of nearly 500 species. The chromosome number is known hardly for 82 species (vide Table 1 + present reports), of which 53 have shown somatic count as 2n=42. Darlington and Wylie (1955) and Love and Love (1961) suggested \( X = (7) \? 21 \) as the basic number for the genus. Mehra and Saha (1970) on the basis of 2n=28 in \( \text{H. oscitifera} \) and \( \text{H. oldhami} \) (Miduno, 1939, 1940) and normal meiotic course of the species in their investigation with \( n=21 \), also arrived at a conclusion that \( X_1 = 7 \) is a base number for the genus. Majority of Habenaria species investigated (65%) so far are at the hexaploid level of \( X = 7 \). \( \text{H. decaplena} \), \( \text{H. longicornu} \) and some others are occupying 12-ploidy level with 2n=84. \( \text{H. reniformis} \) (2n=112), \( \text{H. pliancucena} \) (2n=126) and \( \text{H. geniculate} \) (n=84) are of 16-ploid, 18-ploid and 24-ploid respectively on this base number.

Mehra and Vij (1972b) opined that the presence of chromosome numbers such as n=16, 20, 24 and 2n=32, 40, 48, 64 in some species of the genus (Sampathkumaran and
pose difficulty in considering 7 as the only base number for the genus. For this reason they proposed another base number $X = 8$ for Habenaria. Later report of $n=20$ in H. josephi Rehb. (Vij et al., 1976) adds to this observation. Gametic numbers like $n=22, 23$ and somatic numbers like 38, 44, 46, 62, 108 are the aneuploids at the hexaploid or higher level either on $X = 7$ or 8. In view of the somatic chromosome numbers such as $2n=42$ (H. heyneana, H. longicorniculata and H. subpubens) and $2n=84$ (H. cerea and H. cerea var. polyantha) obtained in the presently investigated taxa, the primary base number $X_1 = 7$ is supported. The regular formation of bivalents further suggests the secondary base number to be $X_2 = 21$.


This genus contains about 80 species distributed in the Northern Temperate zone of the world particularly in North America; a few species occur in Europe and Tropical Asia. Twenty one species have been reported so far for the chromosome numbers. Fifteen of them have shown somatic number as $2n=42$. Two species are found with intraspecific polyploidy, one each with $2n=16$, 63 and 126 and a report
of varying chromosome number in single species. In the present study Platanthera susannae (L.) Lindl. shows the normal somatic number as 2n=42. This report joins the series of species with 2n=42 of the genus having X₁ = 7 (Duncan, 1959) as the primary base number.

Peristylus Bl.

The genus Peristylus Bl. is represented by about 80 species (Bentham and Hooker, 1889). In spite of the interest provided by the wide distribution of this genus and effective vegetative means of propagation, no karyomorphological work has yet been done. The first chromosome report on the species of this genus is made by Larsen (1960) in P.cordatus Link. as 2n=36. After that, until now, chromosome numbers of only 3 more species of this genus could be added. Arora (1968) reported gametic number as n=21 for P.goodyeroides from Western Himalaya. Later, Mehra and Vij (1970) recorded n=21 and n=23 for P.aristatus and P.goodyeroides respectively. Recently Mehra and Seghal (1974, 1980) have reported somatic counts for P.aristatus, P.stenostachyus and P.goodyeroides as 61, 42 + 2B, 46 + 1-3B respectively.

In view of the scanty information about chromosome number and lack of detailed karyomorphological work in the...
Fig. 1. A probable line of evolution of the three genera in the subtribe Orehidinae. Available reports of chromosome number classes are given in their increasing order, and distance between the two numbers does not relate to frequency of a particular class. However, chromosome numbers in the species of the three genera are relatively on very high frequencies at 2n=42 in Habenaria Willd., 2n=42 in Platathera L.C.Rich. and 2n=46 in Peristylus Bl.
members of \textit{Penstylus}, proposal for its base number could not be made.

Seven, out of eight, species of this genus in the present investigation show somatic count as $2n=46$. This seems to be an aneuploid number at hexaploid level on $X_1=7$. However, from the uniform number of $2n=46$ noted for the 7 species of \textit{Penstylus} it can be inferred that there has been a deviation in this group from its allied genera viz., \textit{Habenaria} Willd. and \textit{Platanthera} L.J.Rich., pretty long back in the history of evolution as an aneuploid offshoot (Pl. XXII, Fig. 1).

Twenty three bivalents have been observed clearly in the species which could be studied for their meiosis. Such a regular constancy in chromosome number with extensive vegetative propagation is quite remarkable. However, the explanation of such a stability possibly lies in the fact that in addition to the vegetative propagation, sexual reproduction in Orchids is highly advanced and specialized. In the absence of any peculiarity in chromosome behaviour, the 23 chromosomes may for the present be considered as not only haploid number but even the basic set for the genus (Secondary base number $X_2 = 23$). Reports of $2n=56$ by Larsen\cite{Larsen} in \textit{P. cordatus} Link. and $2n=40$ in one species of the present
The above table shows the quantitative distribution of species among all the chromosome number classes known for the given genus, as well as the number of species with intra-specific polyploidy (ISP) and with a different chromosome numbers (DCN). Numerical in parenthesis specify the number of species for particular chromosome class found in the present work.

<table>
<thead>
<tr>
<th>Genus</th>
<th>2nd numbers</th>
<th>2nd numbers</th>
<th>2nd numbers</th>
<th>Total species</th>
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<tbody>
<tr>
<td>Habeneria</td>
<td></td>
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<tr>
<td>Love &amp; Love (1961)</td>
<td>28, 32, 38, 40, 42, 44, 48, 64, 84, 108, 112, ISP, DCN</td>
<td>1, 3, 1, 1, 53, 5, 1, 1, 4, 1, 1, 3, 7, 82</td>
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<td>Nehra &amp; Bawa (1970)</td>
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<td>Love &amp; Love (1961)</td>
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<td>2nd numbers</td>
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<tr>
<td>Distanthera</td>
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<tr>
<td>Dunson (1959)</td>
<td>16, 42, 63, 126, ISP, DCN</td>
<td>1, 15, 1, 2, 1, 21</td>
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<tr>
<td>X = 7</td>
<td></td>
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<tr>
<td>Peristylus</td>
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<tr>
<td>X = ?</td>
<td>36, 40, 42, 46, DCN</td>
<td>1, 1, 5, 2, 10</td>
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investigation may be considered as isolated cases of aneuploidy.

(ii) **Significance of structural alterations in speciation**

Structural specificity of chromosomes is of prime importance in deciding the individuality of a taxon. A comparative study of the karyotypes of different species has not only provided the information on the trend of evolution of orchid species but has also revealed the interrelationships between them. The significance of structural alterations of chromosomes in evolution of orchid species has been pointed out by Sharma and Chatterji (1966), Jorapur (1968), Wilfert and Kamemoto (1969), Tara and Kamemoto (1970), Roy and Sharma (1972), Jorapur and Hegde (1975), Jorapur and Kulkarni (1973) and others.

The importance of structural alterations of chromosomes in the evolution of species can only be ascertained in cases where more than one species have been investigated in a genus. Such genera in the present work are *Habenaria* and *Peristylus*. In the species of the genus *Peristylus*, structural changes of chromosomes have been very well correlated with lip specialization. In the other genus
Fig. 1  Histogram showing total chromosome length in members of orchidinae

PLATE xxiii

- P. plantagineus
- P. elatus
- P. goodyeroides
- P. lawii
- P. paristatus
- P. stocksii
- P. densus
- P. xanthochlorus
- H. heyneana
- H. subpubens (2n = 42)
- H. subpubens (2n = 44)
- H. cerea
- H. cerea var. polyantha
- H. longicorniculata
- Platanthera susannae
Habenaria however, the gross idea of differences in karyotypes at species level is perceived.

Of Habenaria, five taxa have been investigated. Three of them are showing somatic number as 2n=42 and other two as 2n=84. The size of the chromosomes noted in H. hoyneana is rather long as compared to other species investigated here. All the five species show graded karyotype and a slow diminution in total chromosome length. The histogram (Table 23; Pl.XXIII, Fig.1) shows that in H. hoyneana the total chromosome length is the highest (93.15 μm) and is gradually reduced from H. subpubens (65.61 μm) to H. longicorniculata (63.05 μm). In collection number 2511 of H. subpubens the somatic chromosome number is found to be 2n=44 with total chromosome length of 72.09 μm. It seems that the increase in total chromosome length in the above collection, compared to the total chromosome length of normal complement with 2n=42, is due to 2 additional submedian short chromosomes.

H. cerea and H. cerea var. polyantha, with higher ploidy level (2n=84) have total chromosome length as 130.95 μm and 125.82 μm respectively. Such a steady diminution in total
chromosome length associated with evolution has been reported in other species of flowering plants (cf. Stebbins, 1950, 1971) and in orchids as well (Sharma and Chatterji, 1966; Roy and Sharma, 1972; Jorapur and Hegde, 1975; Jorapur and Kulkarni, 1979). In addition to this progressive diminution, chromosome length and morphology as well have undergone rearrangement in different species studied here (vide respective karyograms and Table 23). These evidences demonstrate clearly that chromosomal changes in structure along with polyploidy have profoundly affected the evolution of species in the genus Habenaria.

Another point of interest here is the status of Habenaria subpubens A.Rich., H.cerea Blatt. & Mc. and H.cerea var. polyantha Blatt. and Mc. on biosystematic considerations. Habenaria subpubens A.Rich. has been treated as a synonym of H.hayneana Landl. by Gamble (1928) as well as separate species by Hooker (1890) and Cocker (1908). Blatter and McGann (1932) while revising the flora of the erstwhile Bombay Presidency considered H.subpubens as a synonym of H.hayneana but created a new species H.cerea sp. nov. (Type locality - 1st Tableland, Panchgani) and its variety H.cerea var. polyantha var. nov. (Type locality - 3rd Tableland, Panchgani). Recently, Santapau and Kapelia (1966)
reduced the above mentioned four taxa as one under the old and legitimate name of *H. heyneana* Lendl. In the present work, the plants of *H. heyneana* Lendl. and *H. subpubens* A.Rich. are studied from their described localities and *H. cerea* Blatt. & McO. and *H. cerea* var. *polyantha* Blatt. & McO. from their type localities. On the basis of overlapping morphological characters, similar somatic numbers (2n=42) and dissimilar karyotypes in *H. heyneana* Lendl. and *H. subpubens* A.Rich., it is inferred that the two are cytotypes.

However, position of *H. cerea* Blatt. & McO. as an independent species is quite clear by virtue of its distinctive morphological characters, allopatric distribution and higher ploidy level (2n=84). Variety *polyantha* Blatt. & McO. has not shown any character which can make it distinct from its type i.e., *H. cerea*. The chromosome number in the variety is also 2n=84 but the karyomorphology is found to be different. This indicates that *H. cerea* var. *polyantha* is a cytotype of *H. cerea*.

**Peristylus Bl.**

The study of karyotypes of eight species of the genus *Peristylus* has yielded certain facts of fundamental importance. There is undoubtedly a general similarity in the gross morphology of the chromosomes. All the species
are characterised by graded karyotype. This certainty suggests the homogeneity of this group. In their minute details, however, specially in the number of different categories of chromosomes and in the precise location of primary constrictions, each species is distinct from the other. This confirms not only the specific status of the taxa but also indicates that continuous accumulation of structural changes has mainly been responsible for the origin of new species. Such an observation is also made by Sharma (1956), Sharma and Sharma (1959) who emphasize that structural alterations have more chances of survival compared to numerical variations in the species reproducing profusely through vegetative means. Sen (1973) has claimed that strains and species would differ in relation to karyotype but not necessarily in number.

According to Stebbins (1950, 1971) primitive species with stable habitat possess more symmetrical karyotype whereas the advanced species have asymmetrical karyotypes. Karyotype analysis of eight Peristylus species show (Table 23) that the chromosomes are mainly medium type. Total chromosome length and number of long, medium and short chromosomes do not bring out any evolutionary relationship. However, chromosome type in relation to centromere location is found to give some clue regarding evolution in the 8 species of
the genus presently studied. In the evolutionary line up, *P. plantagineus* comes at the base as revealed by its comparatively primitive karyotype. It has maximum number (24) of median chromosomes along with 22 submedian chromosomes. Subterminal chromosomes are not encountered in the karyotype. This fact is also evidenced by its simple petal like lip. *P.elatus* occupies the next position in evolution which is clear by an increase in the number of submedian chromosomes (26) and presence of 4 subterminal chromosomes in the karyotype. *P.goodyeroides* also has 4 subterminal chromosomes but the lip in *P.goodyeroides* has shown the advanced feature. This suggests that *P.elatus* and *P.goodyeroides* are later derivatives of *P. plantagineus*. Correlating with the modification of lip from *P.goodyeroides* towards *P.lawii* it is interesting to note that in *P.lawii*, the number of subterminal chromosomes is increased to 8 with the decrease in the number of submedian chromosomes. This incidently suggests that the direction of evolution of *P. plantagineus* to *P.lawii* has been from symmetry to slightly asymmetry. When *P.lawii* and *P.stocksii* are considered comparatively, great increase in the number of short chromosomes is noted in the latter. Except 4 median chromosomes, other chromosomes are submedian. No chromosome is found to have subterminal centromere. Total chromosome
length is drastically reduced which not only confirms its higher level of evolution but also indicates that the direction of evolution of chromosomes in this taxa has stuck up at submedian chromosome stage. All this data obviously strengthens the advanced position of *P. stocksii*. The karyotype of *P. aristatus* shows 2 subterminal chromosomes, and the size difference in the chromosomes is comparatively more. The somatic chromosome 2n=40 indicates that it is a case of aneuploid deviation. However, in lip structure, *P. aristatus* attains the same level of advancement as that of *P. xanthochlorus*. The last two species viz., *P. densus* and *P. xanthochlorus* establish their advance status over all the species studied. *Peristylus xanthochlorus* seems to be comparatively advanced by showing higher number of submedian chromosomes, of which 6 are of short type. In *P. densus* only 2 short chromosomes are noticed. The total chromosome length of *P. xanthochlorus* is less (61.52 µm) than *P. densus* (64.25 µm). Further, presence of a pair of SAT-chromosome in the former shows its active nature.

Thus the above karyomorphological observations in the eight species of *Peristylus* reveal a line of evolution (PI.XXI, Fig.1) which coincides with the line that has already been drawn on the basis of lip specialization (l.c.).
(iii) Polyploidy and concept of species

Formation of polyploids in nature is an important and well-established evolutionary mechanism. On the basis of the nature and degree of morphological differentiation involved, polyploids are divided into cryptic, semicryptic and distinct, and the consequent taxonomic status has been afforded to them ranging from specific rank to no formal recognition at all. Some cytotaxonomists believe that every polyploid level should be treated as a distinct taxonomic species. This attitude is based mainly on the assumptions that chromosome evidence is more important than morphological evidence and that polyploidy causes the immediate separation of an effectively isolated unit or gene pool (Valentine, 1949, Valentine and Löve, 1958). Söcher (1961) remarked that it is not the doubling up of a chromosome complement which is crucial but rather the success of polyploids. If they are able to find a separate ecological niche, isolation mechanisms will soon remove them from their diploid ancestor(s). Therefore, for a species criterion, polyploid must exist as an independent differentiated population. According to Löve and Löve (1961) all the taxa differing in chromosome number in related forms should be regarded as distinct species. However, Smith (1933) and Smith-White (1954) are among those who advocate against the reliance merely upon
chromosome numbers for taxonomic divisions since it is incompatible with the system of natural classification. Manton (1934) and Davis and Heywood (1963) remark that recognition of polyploid at specific rank is appropriate provided that morphological characters separating them are unambiguous. Mehra and Vij (1972a) comment that, already the various synonyms used by different authors have intricated the nomenclature in Orchid family. Species or subspecies based merely on chromosome numbers, stomatal size, number of leaves or flower colour are liable to create taxonomic confusion in a family like Orchidaceae which is enriched with hybridization and is considered to be in an active state of speciation. Further they remark that polyploids should be considered only as forms of the same species and no different name or status be conferred upon them, unless these are accompanied by certain well marked morphological characters making them fairly distinct from the parent species and easily recognisable in the field. The presently investigated taxon from 1st Tableland, Panchagani, described as *H. cerea* sp. nov. Blatt. & McC., is a case here in consideration. These plants have shown distinct morphological characters (l.c.) with somatic chromosome number as 2n=84. *Habenaria heyneana* Lindl., with which the above taxon is merged (Santapau and Kapadia, 1966),
has somatic chromosome number $2n=42$. Plants of *H. cerea* sp. nov. of Blatter and McCann are homogeneously spread on the Tableland in spite of the extreme biotic disturbances and climate conditions. The somatic chromosome number $2n=84$ is a clear indication that the population has developed through polyploidization. This phenomenon must have facilitated more intensive partitioning of habitat into specific ecological niche during saturation and isolation process of this 'now established' species. However, plants of 3rd Tableland described as variety *polyantha* var. nov. by Blatter and McCann on the basis of merely characters like double the size of flower parts etc. seems to be unround. In the present investigation, even these characters are not observed uniformly in all the plants of 3rd Tableland (l.c.). The somatic chromosome number of this taxon is also $2n=84$, but the karyotype is different. Keeping these points in view, it will be justifiable to consider the population of 3rd Tableland as cytotype of *H. cerea* Blatt. and McC. (Plants of 1st Tableland) on biosystematic considerations.

(iv) Aneusomy and speciation

In the present study, *Peristylus densius*, *P. goodyeroides*, *Habenaria cerea* var. *polyantha* and
Plat anther a susannae have shown variation of chromosome number in somatic tissue in addition to the normal number. In such cases, the number occurring in the highest frequency is taken as the normal number for particular individual.

In recent years such variations of chromosomes in the somatic cells along with the normal ones have been recorded in a large number of species reproducing through vegetative means (Sharma, 1956; Sharma and Bhattacharyya, 1956; Sharma and Hal, 1956; Sharma and Sharma, 1959; Sen, 1973). Further, it has been demonstrated that an abnormal number in one species was the normal number of another species (Sharma and Sharma, 1959). Therefore it was suggested by the above workers that if an abnormal nucleus with a numerically or structurally altered karyotype enters into the growing apex, which gives rise to a daughter shoot through vegetative means, the new shoot will be formed of the cells containing the altered number. Such new shoots will evidently differ both phenotypically and genotypically from the original individual and new forms may originate through somatic mutation. In this reference, the variation number 2n=36 chromosomes in Tania minor and the normal number 2n=36 chromosomes in Tania laxifolia have already been emphasised (Sharma and Chatterji, 1966). Another similar case to cite here is the somatic chromosome number.
In most of the species of Vanda so far studied, the chromosome number has been noted to be thirty-eight or its multiple. Significantly the number 36 was also noted by Sharma and Chatterji (1966) in variation nucleus in Vanda teres, V.coerulea and V.coerulescens.

Orchids, though sexually reproduced, are characterised by extensive vegetative reproduction as well. Possibly speciation in Orchids, too, is affected to a significant extent through this process. As such, the inconstancy in the somatic number noted in the present work has an added significance in Orchid speciation.

With regard to the origin of these abnormalities, nothing precise can be stated here. However, in the earlier studies on other plant groups it is inferred that non-disjunction and partial endomitotic reduplication have played a role in polysomaty (Sen, 1973). In the present work the decreased chromosome number in aberrant nucleus such as 2n=21 and 37 in H.cerea var. polyantha, 2n=23+2 bits in P.goodyeroides and 2n=36 in Platanthera susannae might have arisen either due to nonduplication of mitotic chromosomes in the division previous to it or centric fusion as demonstrated by Jones (1978) or by the loss of chromosomes.
due to unequal translocation as described by Stebbins (1971).
The aberrant number \(2n=44\) in a cell of *Platanthera susannae*
seems to be a case of partial somatic reduplication. This way aneuploidy may originate in the species of this genus.

A point can be raised against non-disjunction as a possible means for the origin of such variations. In all the cases cited in literature, no specific zonation of their occurrence has been found. If the origin of these abnormalities through non-disjunction is assumed to be correct, it is then also expected that at least in some cases the two compensatory numbers, one showing deficiency and other showing an increase, would be found side by side. However, no such report is recorded. Though simultaneous occurrence of normal and abnormal numbers has been observed in *Peristylus densum* (Pl. IX, Fig. 5) of the present study where the two cells are lying side by side, yet the numbers are not compensatory. Another point is worth mentioning about the presence of cytotype (Coll. No. 2514) with somatic number \(2n=44\) in *H. subpubens*. The normal somatic number of *H. subpubens* is \(2n=42\). It seems that in the cytotype this aneuploid number has arisen through aneusomic cell where partial somatic reduplication has occurred in the complement.
(v) **Chromosome behaviour at meiosis**

Valuable information can be obtained from chromosomal behaviour at meiosis. More frequent use is made of meiotic behaviour as a means of indicating relationships through the kind and degree of pairing. The chromosomes derived from the two parents are often not homologous at meiosis and do not therefore pair. However, it has been shown in *Triticum* that chromosome pairing can be under relatively simple genetic control (Riley, 1960). Meiotic behaviour in all the species studied under investigation is mostly regular. However, a few irregularities like precocious movement of chromosomes, laggards, stickiness etc., are noted. Though majority of the nuclei of PMCs show uninucleolate condition, binucleolate condition has also been observed frequently. Secondary association is observed only in one species viz., *P. goodyeroides*. In *P. densum* and *P. xanthochlorus*, 1-2 chromosomes are observed to be regularly precocious. Occasional laggards have been noted in *H. subpubens*. The finding of such an abnormal behaviour may be due to environmental causes (Swanson, 1963) and might induce a genetic imbalance affecting the phenotype of the species. Another type of abnormal behaviour is the formation of anaphasic bridges at metaphase I as observed in *P. goodyeroides* and stickiness.
noted in *H. subpubens*. This may be attributed to the homologies that exist among the chromosomes. Pollen mitosis has been studied in *P. aristatus, P. denue, P. gooderoides, Habenaria ceras var. polyantha* and *Platanthera susannae*.

Five species of *Habenaria* studied here, show regular course of meiosis. In *H. longicovinulate*, twenty one bivalents have been observed clearly. This fact is taken into consideration in supporting the primary base number $X_1 = 7$ and to put forward the view that the secondary basic number of the genus is $X_2 = 21$.

(vi) **Characteristics of microsporogenesis**

In *Habenaria subpubens, Peristylus gooderoides* and *P. xanthochlorus*, the first division of the male gametophyte (Pollen grain) has been noted to occur while still inside the anther. This has been seen in other Orchid genera as well (Barber, 1942; Sharma and Chatterji, 1966 and others). At the time of division, the microspores do not separate and remain in a tetrad condition, so that after division, it simulates the appearance of an octant. The peripheral nuclei in the octant are more deeply stained than the other ones. The wall separating the peripheral nuclei is transitory and soon disappears. At this stage of binucleate quatro
pollen grains, usually uninucleolate nature is seen, however, quite often many nucleolate nuclei are also encountered.

Microspore division has been found to be simultaneous. This mode of microspore division which is an indication of advanced stage in evolution also confirms the highly evolved status of orchid genera.

(vii) **Significance of secondary association observed in P. goodyeroides**

Secondary association of bivalents are now considered to represent homology between associating members not sufficient enough to ensure pairing. In this phenomenon, the bivalents are associated in groups of different numbers at diakinesis and first metaphase and even persisting in some cases up to second metaphase.

Presently, in one species of *Peristylus* viz., *P. goodyeroides*, this association has been observed (Pl. IV, Fig. 3). Twenty three bivalents are found associated into 7 groups in many cells. But this association did not cause any irregularities in the subsequent stages of meiosis.

Since its first detection in *Oryza sativa* by Kurosawa (1910), secondary association has been noted in number of species. Secondary associations have been of wide occurrence.
m polyploids (Lawrence, 1931) and these generally do not involve more chromosomes than the degree of polyploidy in orchids (cf. Duncan, 1959). However, their occurrence has been reported in haploids and diploids in this group of plants (Miduno, 1940). Many authors accept that secondary associations indicate evolutionary affinities among the chromosomes concerned and they are favoured by residual attractive forces acting between the once homologous chromosomes in ancient polyploids. Riley (1960) demonstrated by the use of cytological markers that in *Triticum aestivum* equivalent bivalents from different genomes occurred in close proximity at metaphase. Nishiyama (1956) suggested that the original base number of the species could be ascertained from the secondary pairing of chromosomes. In counting the basic number from secondary association, two types of counts have been suggested - (a) the association that occurs in the maximum frequency, or (b) the minimum number of groups present showing maximum association. The latter method seems to be correct because of the homology between the bivalents is not enough to overcome all intracellular barriers which lie between their association in all cases (Sharma, 1976). For Orchids, on the basis of these associations, Sharma and Chatterji (1966) have suggested 10, 7, 9 and 9 as the basic numbers in *Temia*. 

Phalaenopsis, Vanda and Rhynchostylis respectively. Duncan (1959) and Mehra and Vij (1972) have however, voiced against the reliance upon secondary associations for deducing the basic numbers. Various other causes have been ascribed for secondary associations. Some authors feel physical or chemical factors of chromosomes to be responsible. Thomas and Ravel (1946) demonstrated that the heterochromatic regions have a tendency to associate with similar ones at meiosis in Cicer arietinum. The probable cause of these associations, related to the activity of ribonucleic acid, emerges out from experimental studies of Johnson (1951) on the root tips of Paphiopedilum insigne sylhetense. Unfavourable temperature and habitat conditions in which plants grow are also reported to be one of the causes (cf. Kobayashi, 1952). According to Btiflau – Reim (1943), these associations in Orchids are the consequence of the presence of mycorrhizal fungus.

If the theory of secondary association of bivalents as a means of finding out the basic number of a species is considered to be correct, then secondary association of bivalents in P. goodyeroides is a point of interest. In the species, the least number of groupings has been found to be 7 which coincides with primary basic number of the group proposed by earlier workers (l.c.).
Accessory chromosomes

Some cells of collection number 2595 of *Habenaria heyneana* and coll.no.2516 of *H. longicorniculata*, in the present study, have shown the presence of 2 accessory chromosomes each.

Accessory chromosomes were first observed in the insect genus *Diabrotica* and were first detected in plants in *Zea Maize* (Longley, 1927; Randolph, 1928 and 1941). Since then, there have been numerous reports of their occurrence (Cleland, 1951; Lewis, 1953; Reese, 1954; Bosemark, 1956a, b and 1957; Frost, 1956, 1958a, b). Reviews of nature, origin and significance of accessory chromosomes are given by Darlington (1956), Muntzing (1957 and 1959), Larsen (1960a), Battaglia (1964) and Jones (1975). Duncan (cf. Withner, 1959) noted that accessory chromosomes have not been identified with certainty in orchids. However, there are few reports of their occurrence in the members of Orchidaceae as in *Cypridium acaule* (Belling, 1924), *Listera ovata* (MacMohan, 1936), *Epipactis latifolia* (Hagerup, 1977), *Paphiopedilum insigne* (Duncan and Macleod, 1948), *Saimis laxifolia* (Tanaka and Matsuoka, 1972).

Of the subtribe Orchidinae, *Habenaria atropetalschys* (Mehra and Seghal, 1974), *H. pectinata* (Mehra and Kashyap,
1976) *H. acuifera* and *Peristylus goodyeroides* (Mehra and Seghal, 1980) are on the record for the occurrence of B-chromosomes.

The chromosome number variation by supernumeraries in *Epipactis latifolia* and *Listera ovata* has been interpreted to be perhaps, leading to the polysomic condition. B-chromosomes in *Paphiopedilum wardii* has been found involved in aneusomy (Duncan, 1945). Literature reveals that the principal effects of accessory chromosomes are on the overall vigour of the plants as well as pollen fertility (cf. Stebbins, 1971 and Sharma, 1976). It is also suggested that the lesser number of accessory chromosomes may increase variability and thereby adaptability (cf. Davis and Heywood, 1967). A lack of correlation between morphological traits and number or presence of B-chromosome is a frequent result of many investigations. In the present study, however, phenotypic differences have not been encountered between the carrier plant and non-carrier plants.